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THE REMOVAL OF WALL DEPOSITS BY HIGH FREQUENCY DISCHARGES[1]

BY R. H. HAY[2]

Abstract

When a high frequency discharge is maintained between external sleeve electrodes in a tube coated with a thin metallic film, the film is removed from some regions and deposited in others, to give patterns which vary with frequency, pressure, the nature of the gas carrying the discharge, the nature of the film, and the tube diameter. Frequencies of 3.0, 60, and 300 megacycles per sec. have been used with discharges in air, nitrogen, oxygen, and hydrogen, at pressures ranging from 0.07 to 3.5 mm. of mercury and with coatings of silver and, in some cases, of sulphur. Tubes of 18 and 38 mm. outside diameter were used with a standard electrode spacing of 8 cm. and an electrode width of 1 cm.

Evidence is given that the removal is of two types: (i) local, taking place underneath the electrodes; and (ii) general, occurring adjacent to the positive column of the discharge. The cause of both types is found in the bombardment by positive ions: the local, or electrode, effect varies inversely as the pressure and the frequency; the general removal is independent of frequency, but varies directly as the pressure and the gas ion mobility, and inversely as the radius of the tube.

In interpreting the results, use is made of the hypothesis that the high frequency discharge is in effect two d-c. discharges with the electrodes as cathodes and the positive column as an effective anode. Verification has been found in the alteration of the positive column, the Faraday dark space, and the negative glow, with pressure variation and the variation of electrode spacing, and in the reaction of the negative glow to a transverse magnetic field.

Deposition regions are identified with the intersection of the negative glow and the tube wall, but no satisfactory explanation is offered for the fine structure sometimes observed in such regions.

Introduction

In 1933, C. W. Clapp, working in the laboratory of Prof. J. K. Robertson, observed that the high frequency discharge induced by sleeve electrodes removed a previously deposited metallic coating from those portions of the tube walls which lay beneath the electrodes. Subsequently, Robertson removed the coating from the entire inner surface of a tube by moving the electrodes from one position to another, using a tube the coating on which had resisted the action of a hot flame.

In a preliminary investigation of this phenomenon, Robertson and Clapp (13) subjected glass tubes, lightly silvered on the inner walls, to high frequency discharges at various pressures and at a constant frequency. They found that there was evidence of a distinct removal pattern which depended upon

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the nature of the discharge, the time of exposure, and the temperature of the tube, and that patterns could be obtained by silvering a tube after it had been subjected to the discharge.

At about the time that Robertson and Clapp studied this phenomenon, Banerji, Ganguli, and Bhattacharya (1, 3) observed the formation of regularly arranged deposits in a similar type of discharge tube. After identifying the deposits as mercury vapor that had diffused over from a McLeod gauge, they were led to examine the patterns formed by various substances, some of which were electropositive and some electronegative. Furthermore, they investigated the electric fields in the discharge by means of a modified probe method, and found gradients from tube wall to axis which varied in sign and in magnitude along the axis. Regions in which the gradient is of greatest magnitude were found to correspond to areas where deposits are formed on the wall, the deposits of electropositive materials being in general complementary to those of electronegative materials.

Since Robertson and Clapp had made only a preliminary examination of the phenomenon, and since the explanation of the deposit offered by Banerji and co-workers (1, 3) was not completely satisfactory for some of the removal patterns, the present investigation was undertaken.

Experimental

Three sources of high frequency power were used: a single ended series-fed, 100-watt Hartley oscillator using a Mullard TX3-200 tube; a 40-watt push-pull oscillator using two 304B Western Electric tubes; and a special push-pull oscillator, also using the 304B tubes, built from plans kindly supplied by the Bell Telephone Laboratories, New York. The first oscillator covers, by the use of plug-in coils, the range 1.5 to 12 megacycles per sec.; the second is designed for 60 megacycles; and the third supplies about 7 to 10 watts at 300 megacycles. Frequency measurements were made with a General Radio wavemeter for all frequencies except the highest, for which a Lecher wire system was employed.

Three methods of forming metallic coatings on the inner surfaces of the glass tubes were tried: cathodic sputtering, evaporation, and silvering by means of the Brashear, or Rochelle salt, process. The first two presented difficulties which it was felt could not be overcome successfully in the time at the writer's disposal, so the Brashear process was finally used. Glass tubes, cut in lengths of 16 to 20 in., were thoroughly soaked in warm chromic acid, rinsed in tap water, then in distilled water, and silvered lightly while still wet. The layers of silver, as nearly uniform as it was possible to make them, were of "half-thickness". Too thick a coating is undesirable as it may stop the discharge; a heavy layer also makes it difficult to examine the action of the discharge on the silver. For the most part, the tubes used were of 16 mm. inside diameter although some observations were made with tubes of 34 mm. inside diameter. Some of the tubes were coated with sulphur by passing fumes from melted sulphur through them. Flowers of sulphur condensed on the tube walls and formed a fairly satisfactory layer.

Pressure was measured by means of an insensitive McLeod gauge situated close to the pump and shut off from the discharge tube except when readings were being taken. Pressure in the discharge tubes was regulated by a needle valve placed between the reservoir and the discharge tube. This valve was by-passed by a tap which, normally kept closed, could be opened to wash out the system with the carrier gas. A rotary switch, inserted in the cathode-to-ground circuits of the oscillators, closed the contacts one second out of every two. To minimize thermal effects, an electric fan was used to cool the tubes during exposure to the discharge. When sulphur-coated tubes were used at low frequency and high pressure, the power supply was cut off for 30 to 60 sec. after every few flashes to ensure minimum heat action. Even this precaution was not altogether successful, however, for some of the sulphur tubes show, at low frequency and high pressure, evidence of thermal removal. Tests showed that the glass must begin to soften before heat has any noticeable effect on the silver, and since no tube was allowed to become more than perceptibly warm, heat was not a factor in the formation of silver removal patterns.

The length of exposure to the action of the discharge was usually two to seven minutes, with a few exceptions of twelve and twenty-five minutes. The higher the frequency or the lower the pressure, the longer the exposure necessary to cause visible changes in the silver film. The changes were slightly slower in air than in oxygen and nitrogen; in hydrogen they were slightly faster than in the other gases. Gases subject to the same conditions of pressure and frequency were given equal exposures.

Results

The patterns shown in Figs. 2 to 5 are drawn as longitudinal cross sections of the silver films, black representing silver. All estimations of the amount of removal (or deposit) were made visually. The original thickness of the film is scaled as three-quarters of an inch and the longitudinal scale is slightly reduced from full size. These drawings reproduce, within the limits of the visual method used, the variations in the silver layers on the tube walls.

As the removal patterns seem, in general, to correspond to the different types of discharge observed, these types have been described in detail. There are two broad classifications, a low frequency series occurring at 15 megacycles per sec. and lower, and a high frequency series occurring at 60 and 300 megacycles per sec.

The first series is sketched in Fig. 1 and is composed of six types: *A*, *B*, *C*, *D*, *E*, and *F*. The pressure at which each occurs is given in the figure. The only one for which the pressure is critical is Type *B*, the transition from *B* to *A*, or *B* to *C* being sudden. In all others the transition is gradual, and the division into types is arbitrarily based on the pressure increments. All types occurred in air, nitrogen, and oxygen, the differences between gases being in the colors of the various parts of the discharge. The colors indicated are those observed when air was used. The parts marked "rose" and "pink"

in Fig. 1 generally become yellowish and more brilliant in nitrogen—especially in Types *C* to *F*; in oxygen the luminosity is much less and there are few colors other than blue and rose. Types *D*, *E*, and *F* cause very marked afterglows in all three gases. Exposures in silvered tubes were made, in general, with Types *B*, *D*, and *F*, although silver patterns in air were obtained with all six types. Type *F* is extinguished at a pressure of 10.7 mm. of mercury, while Type *A* continues at the lowest pressure obtainable with the Hyvac pump.

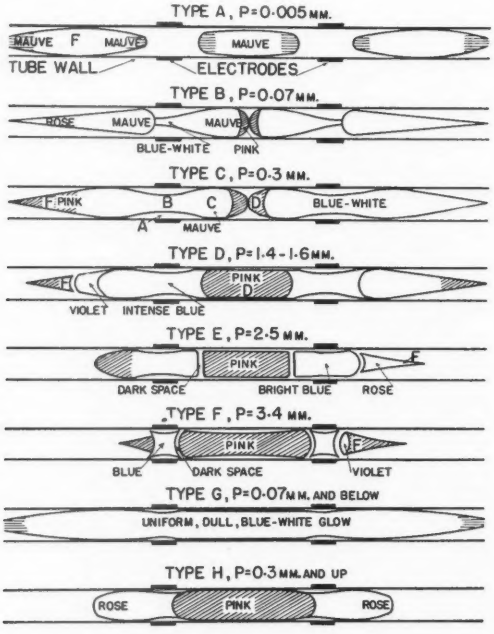

FIG. 1 *Types of high frequency discharge in air.*

As shown in Fig. 1, the high frequency series has but two types. Type *G* (again referring to air) occurs at pressures of 0.005 mm. of mercury and changes gradually, by a shortening of the long central pencils, to Type *H* which continues until a pressure of 2.0 mm. of mercury is reached, when the discharge is extinguished. As in the other series the main difference observable with different gases is one of color, with oxygen less luminous. The 60 megacycle oxygen discharge is extinguished at 0.6 mm. but continues at the lowest pressure obtainable. At 300 megacycles the discharge sometimes must be primed with a momentary discharge from a spark coil or by opening and closing the plate supply switch rapidly. The high frequency series of discharges does not heat the tube as much as the low frequency series, this

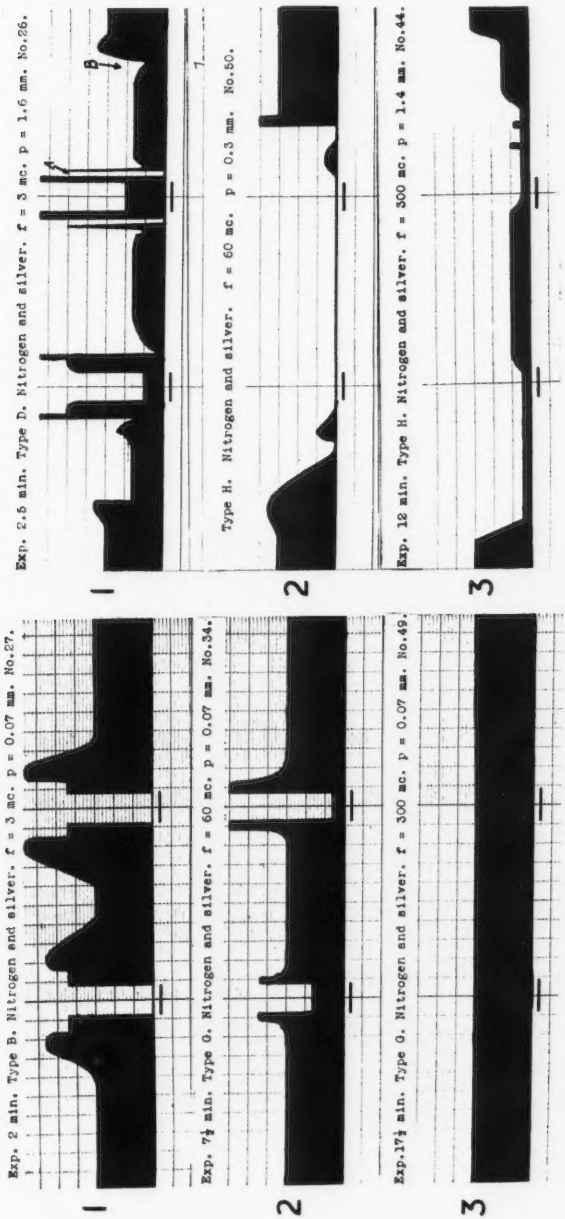
Exp. 2 min. Type B. Nitrogen and silver. f = 3 mc. p = 0.07 mm. No.27.
Exp. 7½ min. Type G. Nitrogen and silver. f = 60 mc. p = 0.07 mm. No.34.
Exp.17½ min. Type G. Nitrogen and silver. f = 300 mc. p = 0.07 mm. No.49.
1
2
3
A
Exp. 2.5 min. Type D. Nitrogen and silver. f = 3 mc. p = 1.6 mm. No.26.
Type H. Nitrogen and silver. f = 60 mc. p = 0.3 mm. No.50.
Exp. 12 min. Type H. Nitrogen and silver. f = 300 mc. p = 1.4 mm. No.44.
B

FIG. 2. *Frequency variation at (A) low pressure, (B) high pressure.*

being due, in part, to the lower power of the high frequency oscillators. Greater heat was developed, in general, at high pressure than at low pressure.

A large number of patterns was obtained, air, nitrogen, oxygen, and hydrogen being used as the carrier gases, at pressures of 0.07 to 3.5 mm. of mercury and at frequencies of 3, 60, and 300 megacycles per sec. Of these patterns, only a few representative ones are reproduced in this paper. While the evidence obtained was not always conclusive, certain main points have been fairly well established:

(1) Removal of silver is of two kinds, that occurring in localized fashion, as under the electrodes (No. 1, Fig. 2, *A*)—*the electrode effect*—and that occurring over large areas of the tube (Nos. 2 and 3, Fig. 2, *B*)—*the general removal*. Both occur simultaneously in some patterns and only one in others. The electrode effect is most noticeable at low pressures and low frequencies. The general removal occurs at higher pressures at all frequencies, and is definitely associated with the pink or yellow glows marked F, in discharge Types *C*, *D*, *E*, and *F*, Fig. 1.

(2) Increase in frequency causes a decrease in electrode effect until it disappears altogether at 300 megacycles. A decrease in pattern complexity is also observed as frequency is increased (Fig. 2).

(3) As shown in Fig. 2, pressure alteration causes variation of the patterns. Pressure increase results in an increase in the general removal and a decrease in the electrode effect. Pattern complexity is greatest at an intermediate pressure and low frequency.

(4) A comparison of silver and sulphur patterns (Fig. 4) suggests that the two substances are complementary in their reactions to the discharge. At high pressure and low frequency, this effect is partly masked by thermal removal of the sulphur, but it is quite apparent at high frequencies and low pressure.

Influence of Carrier Gas

Oxygen and air produced the same effect on silver films as nitrogen. Hydrogen was used with both silver and sulphur coatings, and its effect on the silver (Fig. 5, *A*) is striking in that for all frequencies there is no general removal as there is with heavier gases. Further reference will be made to this point. The sulphur patterns (Fig. 5, *B*) show the effect of chemical removal, as well as electrical effects, for the odor of hydrogen sulphide was very marked at the Hyvac pump, especially during exposures made at low frequency and pressures greater than 1 mm. of mercury. It is interesting to note that removal by chemical combination occurs adjacent to, and at, those places subjected to the electrode effect.

Variation with Width of Tube

A series of exposures was made with nitrogen in wide tubes in order to test certain hypotheses which will be advanced later in the discussion of the general removal. At low frequency, the patterns obtained were similar to

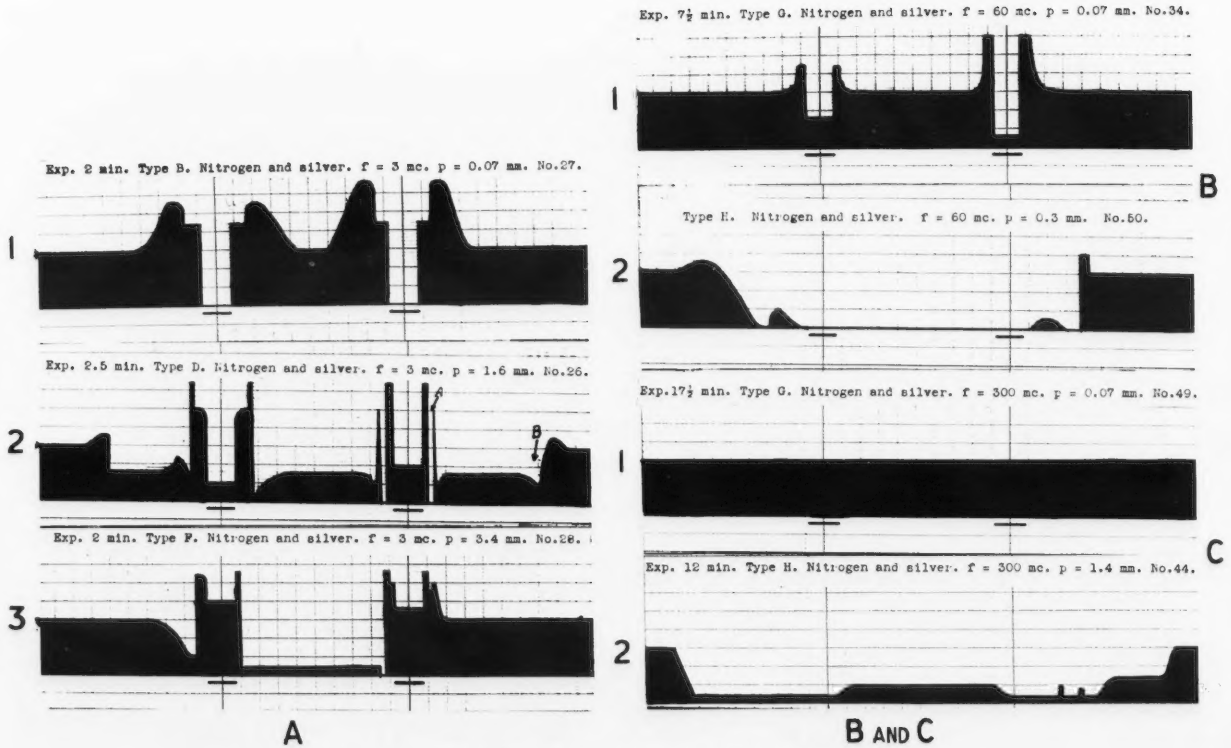
Exp. 2 min. Type B. Nitrogen and silver. f = 3 mc. p = 0.07 mm. No.27.
1
Exp. 2.5 min. Type D. Nitrogen and silver. f = 3 mc. p = 1.6 mm. No.26.
2
Exp. 2 min. Type F. Nitrogen and silver. f = 3 mc. p = 3.4 mm. No.28.
3
A
Exp. 7½ min. Type G. Nitrogen and silver. f = 60 mc. p = 0.07 mm. No.34.
1
B
Type H. Nitrogen and silver. f = 60 mc. p = 0.3 mm. No.50.
2
Exp. 17½ min. Type G. Nitrogen and silver. f = 300 mc. p = 0.07 mm. No.49.
1
C
Exp. 12 min. Type H. Nitrogen and silver. f = 300 mc. p = 1.4 mm. No.44.
2
B AND C

FIG. 3. *Pressure variation at (A) low frequency, (B) and (C) high frequencies.*

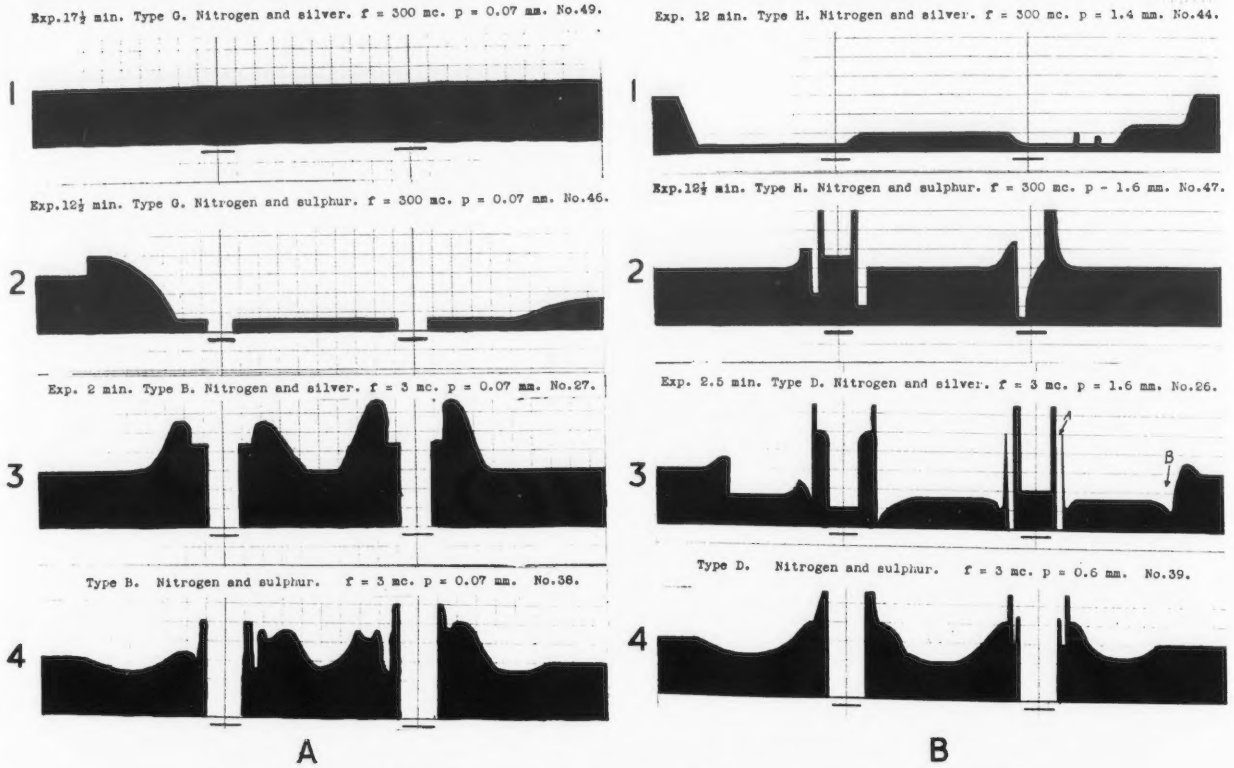
Exp.17½ min. Type G. Nitrogen and silver. f = 300 mc. p = 0.07 mm. No.49.
Exp. 12 min. Type H. Nitrogen and silver. f = 300 mc. p = 1.4 mm. No.44.
1
Exp.12½ min. Type G. Nitrogen and sulphur. f = 300 mc. p = 0.07 mm. No.46.
Exp.12½ min. Type H. Nitrogen and sulphur. f = 300 mc. p = 1.6 mm. No.47.
2
Exp. 2 min. Type B. Nitrogen and silver. f = 3 mc. p = 0.07 mm. No.27.
Exp. 2.5 min. Type D. Nitrogen and silver. f = 3 mc. p = 1.6 mm. No.26.
3
B
Type B. Nitrogen and sulphur. f = 3 mc. p = 0.07 mm. No.38.
Type D. Nitrogen and sulphur. f = 3 mc. p = 0.6 mm. No.39.
4
A
B

FIG. 4. *Comparison of deposit patterns for silver and sulphur at (A) low pressure, (B) high pressure.*

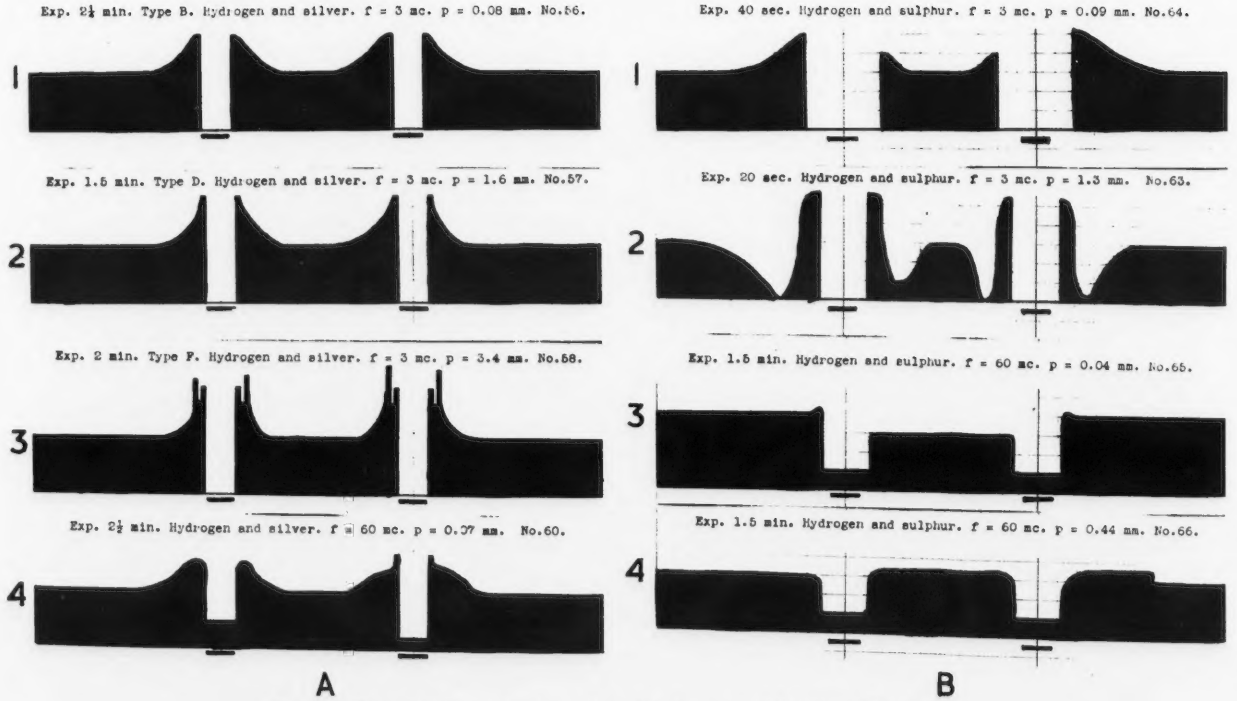
Exp. 2½ min. Type B. Hydrogen and silver. f = 3 mc. p = 0.08 mm. No.56.
Exp. 1.5 min. Type D. Hydrogen and silver. f = 3 mc. p = 1.6 mm. No.57.
Exp. 2 min. Type F. Hydrogen and silver. f = 3 mc. p = 3.4 mm. No.58.
Exp. 2½ min. Hydrogen and silver. f = 60 mc. p = 0.37 mm. No.60.
Exp. 40 sec. Hydrogen and sulphur. f = 3 mc. p = 0.09 mm. No.64.
Exp. 20 sec. Hydrogen and sulphur. f = 3 mc. p = 1.3 mm. No.63.
Exp. 1.5 min. Hydrogen and sulphur. f = 60 mc. p = 0.04 mm. No.65.
Exp. 1.5 min. Hydrogen and sulphur. f = 60 mc. p = 0.44 mm. No.66.
1
2
3
4
A
B

FIG. 5. *Deposit patterns showing action of hydrogen on (A) silver, (B) sulphur.*

No. 1, Fig. 2, *A*, and showed no general removal, no matter what type of discharge took place in the tube. At 60 megacycles per sec., however, a curious reversal of the behavior observed with narrower tubes took place, for at low pressure with a Type *G* discharge a general removal was observed over the region between the electrodes. At high pressure no general removal occurred, the pattern being composed of electrode removal and deposit areas. The Type *G* discharge was observed to fill the tube entirely, leaving only a very thin dark space next the tube wall.

Spectroscopic Observations

In an endeavor to relate the removal pattern with the mechanism of the discharge, a series of spectrograms was taken of various regions of each of the different types of nitrogen discharges. Characteristic results are given in Fig. 6 in which the portions of the discharge associated with the individual spectrograms are clearly indicated. It will be observed in Fig. 6 that the region under the electrode is one of high ionization as indicated by the preponderance of lines and first negative band heads. The deposit region immediately adjacent to the electrode shows a spectrum containing first and second positive band heads and a few first negative heads. The spectrum of the region midway between the electrodes is composed almost entirely of first and second positive band heads. These facts indicate that the region of highest ionization is beneath the electrodes and that as one moves away from there toward the yellow glow in the middle of the tube, ionization decreases and the luminosity of the glow is due to the presence of large numbers of excited molecules.

Spectra taken of discharges at ultra high frequency show the differences between the yellow and the blue glows much more strikingly. When only the blue glow is present (*i.e.*, Type *G* at low pressure) and only the electrode effect is present, the spectra were found to be line and first negative even in the inter-electrode regions. On the other hand, at higher pressures, when the yellow glow (Type *H*) is present, and the removal is entirely of the general type, the spectra show first and second positive band systems for the regions under the electrodes as well as for those between them.

The spectroscopic evidence strongly suggests that the conditions for electrode removal without general removal (Type *G* discharge) are associated with high ionization, while the general removal is linked with a discharge region of low ionization.

Discussion

The Electrode Effect

The experimental results associated with the electrode effect and summarized above suggest that the cause is a positive ion bombardment of the tube walls beneath the electrodes. The effect of such a bombardment on the glass walls has been noted by several workers (14, p. 459) and would explain the observations of Robertson and Clapp (13) that a silver pattern can be obtained by silvering a clear tube after it has been subjected to the discharge. The disintegration of those portions of a paper lining which lay

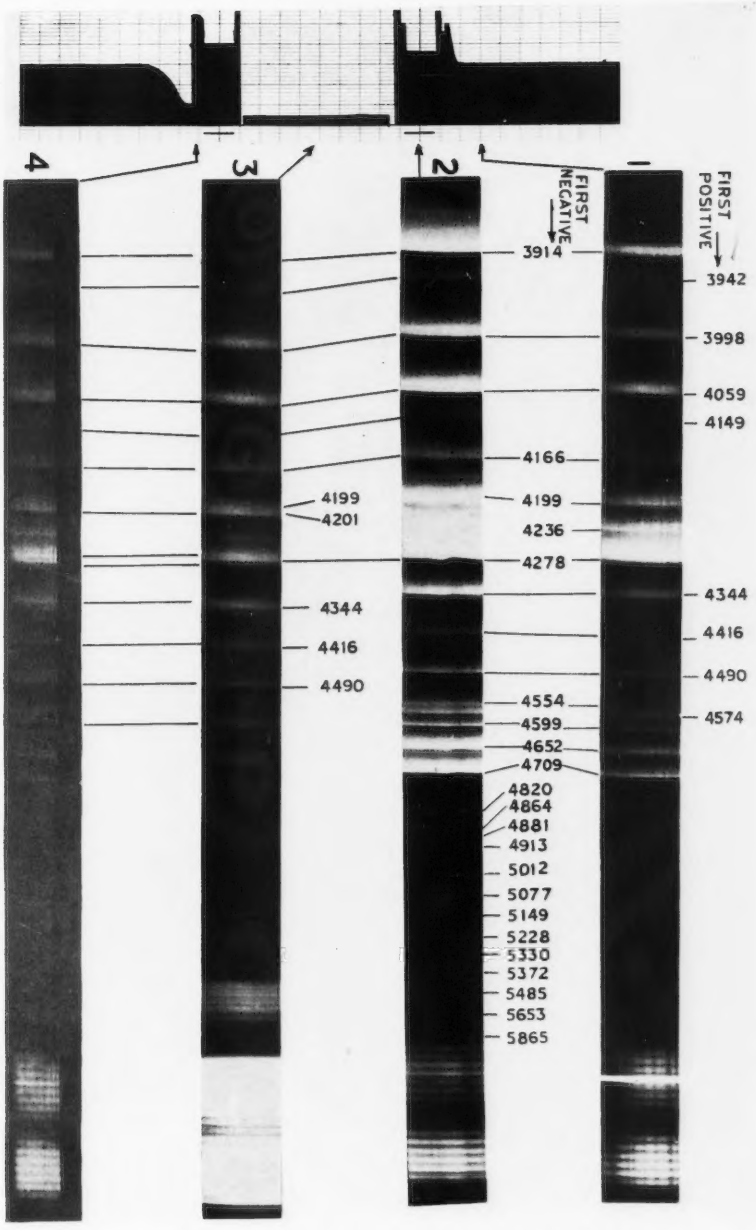

FIG. 6. *Type F. Nitrogen and silver. $f = 3$ mc. $p = 3.4$ mm.*

beneath the electrodes and close to the glass walls is further proof of the existence of such a bombardment.

According to Townsend (17, p. 302; 18), Banerji and Ganguli (2) and others, the high frequency discharge, either with external sleeve electrodes or with internal electrodes, shows alternately every half cycle the same divisions in front of the electrodes as does the d-c. discharge. The high frequency and the greater luminosity at the cathode combine to make each electrode appear to be a cathode. With reference to discharge Type *C*, Fig. 1, A is the cathode dark space and B is the negative glow merging into C, the Faraday dark space, which is succeeded by D, the positive column. The reactions of these regions to the application of transverse magnetic fields, to changes of pressure, and to alteration of the electrode separation were the same as the reactions of corresponding portions of the d-c. discharge.

Assuming such a positive ion bombardment and considering the expressions for maximum velocity and energy, and maximum travel in one-half cycle (which can be obtained by integrating the equations of motion of an ion in an alternating field) one can conclude:

(1) That electrode removal should decrease with increase of frequency,

(2) That at a given frequency (3 megacycles) the removal should decrease with increase of pressure,

(3) That at ultra high frequencies, the maximum half-cycle travel is so small that pressure variation should have no effect on the removal.

Fig. 2, *A* supports the first conclusion, while Fig. 3 shows that the second is true. It is more difficult to support the third, for at ultra-high frequencies and high pressures the electrode effect is masked by general removal.

The same expressions for energy and half-cycle travel suggest that a gas such as hydrogen should be a more active agent than nitrogen. Robertson and Clapp (13) found this to be so, but the results of the present work, while supporting their findings in general, do not indicate differences as great as they observed between the effects of the two gases.

A surprising observation was that d-c. discharges and 60 cycle alternating discharges did not cause any removal of the silver film. A tentative explanation is that at low frequencies the conductance of the thin film is such as to prevent a bombarding discharge. Another possible explanation may be found in the known variation of the dielectric properties of glass with frequency. This change may also be accompanied by changes in the surface structure. However, the unsatisfactory state of the theories of glass dielectrics and the lack of agreement between theory and experimental data make this approach one of extreme difficulty (12, p. 171 *et seq.*).

General Removal

General removal occurs opposite the positive column (D and F, Type *D*, Fig. 1) of the discharge. Of the theories of glow discharges in gases, the plasma theory of Compton, Tonks and Langmuir (4, 10, 11, 15, 16) provides the best explanation for this effect. Treating the positive column as a plasma

we see that there will exist a slow radial drift of positive ions from tube axis to wall, and that at the edge of the plasma, the positive ion sheath, or positive space charge created by the high negative wall charge, may give positive ions energies as high as 13 or 15 volts (10), which have been shown to be sufficient to cause cathodic sputtering of silver (14, p. 458). The smaller the tube radius the greater will be the energies imparted to the ions by the positive ion sheaths.

Consideration of these conditions occurring with plasmas leads to the following conclusions:

(1) General removal should be independent of frequency since the plasma conditions are independent of this parameter.

(2) General removal should occur along any part of the tube that is adjacent to the plasma, and should not be localized.

Fig. 2, *B*, No. 3, Fig. 3, *A*, and No. 2, Fig. 3, *C*, all give strong support to these conclusions.

(3) Patterns obtained with nitrogen in wide tubes should show decrease in general removal; the actual results observed showed no general removal at all.

(4) Owing to the high mobility of the hydrogen positive ion, the action of hydrogen in narrow tubes should be like that of nitrogen in wide tubes. The results shown in Fig. 5, *A*, are striking fulfilment of this expectation.

That the plasma conditions are responsible for the general removal receives further support from low pressure patterns. An electrode spacing of 8 cm. is insufficient to allow the plasma to form in the region between the electrodes at low pressure, but it is formed in the regions beyond each electrode. Silver patterns were obtained which showed general removal opposite such extra-electrode plasmas, so several silvered tubes were subjected to the Types *A* and *B* discharges with the electrodes spaced sufficiently apart to allow the formation of a plasma between the electrodes. These tubes showed plain evidence of general removal opposite all plasmas.

The spectra of nitrogen discharges in the regions of general removal are, almost without exception, first and second positive band systems; this indicates the presence of many excited molecules. The ionization potential of nitrogen is 16.8 volts, while the excitation potentials for the first and second positive band systems are 9.5 and 12 volts respectively (6). The excitation potential for the first negative system is 18.5 volts. The absence of first negative bands and lines in all plasma spectrograms is strong support for the assumption that electron energies are low in the plasmas, and is additional evidence in favor of the view of Langmuir and Tonks (10, 15, 16) that little recombination occurs in the plasma, but rather, at the tube wall in narrow tubes or at the plasma boundary in wide tubes.

Possibility of Chemical Action

The discharges with a positive column, or plasma, were accompanied by afterglows of short duration which, in the nitrogen discharge, may have indicated the presence of active nitrogen. This possibility suggests that at least part of the general removal may have been due to chemical combination between silver and active nitrogen to form silver nitride. Such action is possible (19) but it does not seem at all likely. In the first place, the formation of a layer of silver nitride, which, according to Willey, is inert to active nitrogen, would prevent further action unless one assumes that silver nitride is more easily sputtered than is silver, or that the heat liberated on the conversion of the active nitrogen to the inert variety is insufficient to vaporize the silver nitride. The first assumption is not likely to be correct, since silver is very easily sputtered in nitrogen, but the second is not so easily disposed of, and remains as a possibility difficult either to prove or to disprove. It is conceivable, however, that positive ion bombardment might detonate the silver nitride crystals, thus permitting further chemical action. Attention has already been directed to a removal beneath, and adjacent to, the electrodes, and to the strong odor of hydrogen sulphide at the pump during exposure in a tube coated with sulphur and filled with hydrogen. Chemical combination was undoubtedly responsible for a large part of this removal (See Fig. 5, *B*).

The Behavior of Sulphur

Fig. 4 shows that the action of the nitrogen discharge on silver is opposite to that on sulphur. A possible explanation for this complementary behavior may be found in the differences in the structures of the thin films formed on the glass. The silver was chemically precipitated, was black in color and resisted removal by washing. It formed a layer of small, closely connected crystals fairly firmly bound to the glass. On the other hand, the sulphur layer, formed by the condensation of visible sulphur vapor, could be washed, or even jarred to some extent, from the tube wall; it was, therefore, coarser and much less adherent to the glass.

In the experiment in which silver was removed and sulphur was not, the possible factors causing removal of the silver were positive ion bombardment and chemical action. The same positive ion bombardment of the sulphur layer obtains since the discharges are the same in both experiments. No data are available as to the ease with which sulphur may be sputtered, but its non-removal under low pressure conditions suggests that it is not very great. The discharge has some effect on the sulphur coat, however, for the coarse, yellowish-white layer was altered to a more finely divided, more adherent, yellow, almost glazed coat. A tentative explanation is that electrons reaching the wall are attached to sulphur atoms to form sulphur ions, thus becoming more difficult to remove than are the free electrons on the silver surface. The bombarding positive ions may then recombine on the sulphur surface, the resulting heat of recombination melting the sulphur and reducing the coarse structure to a finer one.

The fact that there seems to be no known compound of sulphur and nitrogen, and that the electron configurations of the two elements render such a combination very unlikely, rule out the possibility of sulphur removal by chemical action. Furthermore, the action of hydrogen on sulphur coated tubes (Fig. 5, *B*) indicates that chemical combination takes place only at those portions of the tube wall which are subjected to positive ion bombardment—*i.e.*, beneath the electrodes or opposite plasmas. Frequency and pressure, which affect the intensity of positive ion bombardment at the electrodes, affect the chemical action as well.

Deposition of Silver Adjacent to the Electrodes

Four points of evidence indicate that the deposition of silver in the regions on both sides of the electrodes is closely connected with the negative glow and the Faraday dark space:

(1) When the negative glow is general, as in Types *B* and *C*, Fig. 1, and the boundary between it and the Faraday dark space is poorly defined, the silver deposit is broad and lacks fine structure (No. 1, Fig. 3, *A*).

(2) When the negative glow is restricted and its boundaries are sharply defined (Types *D*, *E*, and *F*, Fig. 1), the silver deposit is more compressed and possesses fine structure (Nos. 2 and 3, Fig. 3, *A*).

(3) When the cathode head of the negative glow was shifted by the application of a transverse magnetic field, the boundaries of the deposit were shifted in the same manner and by the same amount.

(4) When the silver was removed from half the length of the tube and the electrodes were placed so that the edge of the silvered portion came half-way between them, silver was deposited on either side of that electrode which was over clear glass. Here, too, deposition occurred adjacent to the negative glow.

The formation of fine structure in the deposit areas remains to be explained. Banerji and Ganguli (3) report the existence of limited regions on the tube wall which are positive with respect to the tube axis and which would repel positive ions and thus prevent a deposit of silver. Careful examination might show that these regions (fine structure) correspond to the edges of Faraday dark spaces. Keyston (9) and Tonks and Langmuir (15) report the presence of double negative glows and Faraday dark spaces. These have not been observed in this work, but, nevertheless, they may be present, for in the case recorded by Langmuir they were observed only with the aid of a low power microscope. If more refined methods of observation of the discharge and measurements of the deposit reveal that multiple negative glows and dark spaces exist under the conditions which produce fine structure in the deposit areas, the appearance of a fine structure in the deposit would be explained.

Acknowledgments

The writer wishes to express his thanks to Prof. J. K. Robertson, at whose suggestion and under whose direction this work was carried out, for his kindly and helpful suggestions and for his unfailing interest; and to Prof. H. H. Stewart of the Electrical Engineering Department for his help and advice in the design and construction of the Hartley oscillator.

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A SOURCE OF CADMIUM RESONANCE RADIATION OF HIGH INTENSITY AND SOME PRELIMINARY RESULTS ON THE CADMIUM PHOTOSENSITIZED REACTION OF HYDROGEN AND ETHYLENE[1]

BY E. W. R. STEACIE[2] AND ROGER POTVIN[3]

Abstract

An inexpensive and convenient source of cadmium resonance radiation (λ 3261) of high intensity has been developed. This should make possible more extended studies of cadmium photosensitized reactions. Some preliminary results on the reaction between hydrogen and ethylene are reported.

Introduction

The investigation of mercury photosensitized reactions has proved to be of great interest, since this method makes it possible to endow the reactant molecule with a very definite and reproducible amount of energy. In the case of the paraffin decompositions free radicals are usually produced in the process, and it therefore gives results of special interest in connection with the rates of elementary reactions. The great difficulty here, however, is that hydrogen has a much greater quenching efficiency than have the paraffins, roughly in the ratio of about 50 to 1. Now hydrogen is almost invariably a product of such decomposition reactions. As a result, as soon as a small quantity of hydrogen has built up, the reaction ceases to be the photosensitized decomposition and becomes the reaction of the paraffin with hydrogen atoms.

The $Hg(6^3P_1)$ excitation energy is 112 Kcal. The heat of dissociation of hydrogen into atoms is 102.5 Kcal. The energy required to rupture a C—H bond is about 80 to 90 Kcal., and that for a C—C bond is 70 to 80 Kcal. It follows that if we could obtain a substance with a resonance level about 90 Kcal. above the ground state, the excitation energy would probably be sufficient for the decomposition of the paraffins, but insufficient to dissociate hydrogen, and hence it would be possible to investigate decomposition reactions without complications due to hydrogen atom reactions. There are two metals which have sufficiently high vapor pressures at moderate temperatures, and which satisfy the energy requirements outlined above, *viz.*, cadmium and zinc with $^3P_1$ levels 89 and 92 Kcal. above the ground state respectively.

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, with financial assistance from the National Research Council of Canada.

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[3] *Holder of a bursary under the National Research Council of Canada.*

No previous work has been done on zinc photosensitization, and only one brief investigation of cadmium photosensitization has been made. Bates and Taylor (1) showed that in ethylene-hydrogen-cadmium vapor mixtures illuminated with λ3261 no hydrogenation to ethane occurred, but that some of the ethylene was polymerized. They showed, however, that hydrogen quenched the resonance radiation, and suggested that it took up the energy of the excited cadmium atom as vibrational energy. (Hydrogen has a vibrational level very close to 89 Kcal.) Bender (2) has also investigated the quenching of cadmium resonance radiation by hydrogen.

Bender's results suggest possible complications in both cases, since the reactions

$$Cd + H_2 \longrightarrow CdH + H \ (-86 \text{ Kcal.})$$

and

$$Zn + H_2 \longrightarrow ZnH + H \ (-81 \text{ Kcal.})$$

are possible energetically, and in fact the band spectrum of CdH was detected by him. The extent to which these reactions occur is, however, open to question, and the investigation of cadmium and zinc photosensitized reactions appears to offer interesting possibilities in spite of this disturbing factor.

The above work was done nearly 10 years ago. In spite of its interesting nature it was not followed up, mainly because of the lack of a convenient source of reasonably high intensity in the cadmium resonance line at 3261 Å. As a matter of fact the development of the mercury photosensitization technique was also greatly retarded by the lack of suitable light sources. Recently, however, very convenient high voltage mercury arcs have been developed. These operate from a 5000 volt sign transformer, and employ about 10^{-3} mm. pressure of mercury vapor, with 10 to 15 mm. of neon or some other rare gas as a carrier. In such a lamp no reversal occurs, and the mercury resonance line at 2537 Å is very intense.

A number of lamps have been used in the past for the production of cadmium, or other metal, resonance radiation, neon or other inert gases being used as carriers (3–6, 9–11, 13). Most of these, however, are either weak or unsteady, or else are expensive and complicated and require too much attention to be convenient for photochemical purposes where long exposures are required.

Experimental

To obviate the above disadvantages a lamp has been constructed similar to the existing high voltage mercury lamps (7, 8, 12), the general technique used in neon sign manufacture being employed. A sketch is given in Fig. 1.

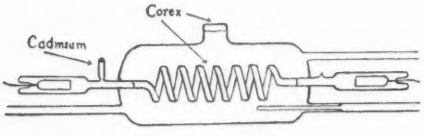

FIG. 1. *Apparatus.*

The emitting part of the lamp consisted of a spiral of 7 mm. internal diameter Corex D tubing. With the wall thickness

employed this has a transmission of about 95% at 3261 Å. Its transmission around 2300 is virtually zero, and it therefore filters out the lower cadmium resonance line. The ends of the Corex spiral were sealed directly to Pyrex tubing of the same diameter. The electrode chambers, also of Pyrex, were 15 mm. in diameter and 7 cm. long. The electrodes were standard neon sign "coated" electrodes 8 mm. in diameter and 3 cm. long. They were welded through nickel to tungsten wires which were sealed through the glass. A small side tube contained vacuum distilled cadmium.

The Pyrex reaction vessel was sealed on as shown. It contained a sealed-in Corex window to permit spectroscopic examination of the discharge. The lamp was filled with neon. The bombarding of the electrodes, filling, etc., were done by the usual technique by the Claude Neon Eastern Ltd.

The whole unit including the electrodes was placed in a furnace which was thermostatically controlled. It was operated at temperatures from 260 to 280° C. (corresponding to cadmium vapor pressures of from 7×10^{-3} to 2×10^{-2} mm.). In the first lamp used the neon pressure with the lamp cold was 15 mm. This gave good intensity but the lamp was very unstable in operation unless quite high cadmium pressures were used (corresponding to temperatures of 280° C. or higher). The lamp was therefore refilled with a neon pressure of 3 mm. This gave much steadier operation at lower temperatures.

The output of the lamp in the region of 3261 Å was measured with a thermopile, and suitable filters, and found to be about 5×10^{-7} einsteins per sec. when the lamp was operated on 80 ma. at about 500 volts. This is within one power of 10 of the output of the most intense commercial high voltage mercury arcs when operated under similar conditions.

A plate taken with a small Hilger quartz spectrograph is shown in Fig. 2. It will be seen that the resonance line at 3261 Å is strong, while the other lines in this part of the spectrum, 3404 Å, 3466 Å, and 3613 Å are relatively weaker. There is very little emission in the visible. The 2288 Å resonance line has been entirely filtered out by the Corex tubing. The lamp therefore seems to be entirely satisfactory for the investigation of cadmium photosensitized reactions.

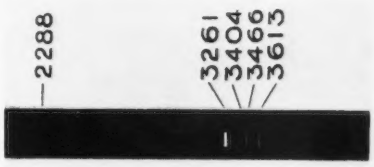

FIG. 2

An investigation of the cadmium photosensitized reaction between hydrogen and ethylene is in progress. Preliminary results indicate that:

(a) With pure ethylene little or no polymerization occurs.

(b) In ethylene-hydrogen mixtures polymerization of ethylene occurs, and in addition there is considerable hydrogenation.

(c) With mixtures high in ethylene the rate of reaction is nearly proportional to the partial pressure of the hydrogen.

The results suggest that either

(i) the reaction

$$Cd(^3P_1) + H_2 = CdH + H$$

occurs to a considerable extent, as has been suggested by Bender (2) so that hydrogen atoms are produced, or

(ii) that we have

$$Cd(^3P_1) + H_2 = H_2^*$$
$$H_2^* + C_2H_4 = C_2H_6, \text{ or } = H_2 + C_2H_4^* .$$

However, the question can be decided only by further work.

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A SULPHUR-CONTAINING SUBSTANCE FROM THE SEED OF *CONRINGIA ORIENTALIS*[1]

By Clarence Yardley Hopkins[2]

Abstract

The seeds of *Conringia orientalis*, which contain a bitter principle, were crushed, freed from fat, and steeped in water. The chloroform extract of the aqueous liquor yielded a crystalline substance which was identified as 2-mercapto-5,5-dimethyl-oxazoline (I). It tastes bitter to some persons but is tasteless to others. The suggestion is made that the seeds contain β-methyl allyl isothiocyanate in the form of a glycoside, and that this substance is converted to (I) during the treatment described above.

Introduction

Conringia orientalis L. (Dumort) or hare's ear mustard is a common weed in the wheat fields of western Canada. Although a number of the Cruciferae have been subjected to chemical examination and have yielded mustard oil glycosides, the components of which are known, this species has not hitherto been studied. The seed has an intensely bitter taste, and is thus unlike the common white and black mustards which owe their burning taste to the isothiocyanate formed by hydrolysis of the sulphur-containing glycoside. It was of interest, therefore, to examine this seed and to determine if possible the nature of the bitter principle. A quantity of the seed became available during the course of another investigation and a study of its constituents was undertaken.

The seeds were crushed and freed from fat by extraction at low temperature with petroleum ether. Analysis of the defatted material showed a sulphur content of about 1%. It had a pronounced bitter taste. Extraction with alcohol in the manner commonly used for isolating glycosides left the residue free from bitterness. The extract yielded a concentrate of the bitter principle in the form of a brown amorphous solid which could not be induced to crystallize, even after varied treatments. Hydrolysis of this substance by acid and by alkali did not give any product that could be characterized.

Further quantities of the fat-free seed were treated with warm water so as to allow enzymic hydrolysis of the glycosides. Extraction of the aqueous liquor with chloroform yielded a crystalline sulphur compound. The quantity of substance obtained was equal to about 0.35% of the weight of the seed. After recrystallization it melted at 108.5 to 109.5° C.*, and analysis indicated the empirical formula C_5H_9ONS.

Preliminary examination of the substance showed that it had none of the reactions of an isothiocyanate. Its properties did not correspond with those of any known substance of the same formula. Experiments were begun to

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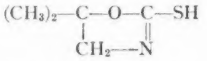
* *Melting points are corrected.*

elucidate its structure and considerable progress was being made when notice of a new substance appeared in the literature, *viz.*, 2-mercapto-5,5-dimethyl-oxazoline (C_5H_9ONS) (1). The melting point and degree of solubility in water, benzene, and petroleum ether were in agreement with those of the compound obtained in the present investigation.

A sample of the synthetic material (I) was obtained, and on examination the two proved to be identical. A mixed melting point showed no depression.



(I)

Further proof of their identity was obtained from measurements of ultra-violet absorption spectra. As shown in Fig. 1 the absorption curves of the natural and the synthetic material virtually coincide. Molecular extinction coefficients were calculated according to the method of Henri (3, pp. 359, 360).

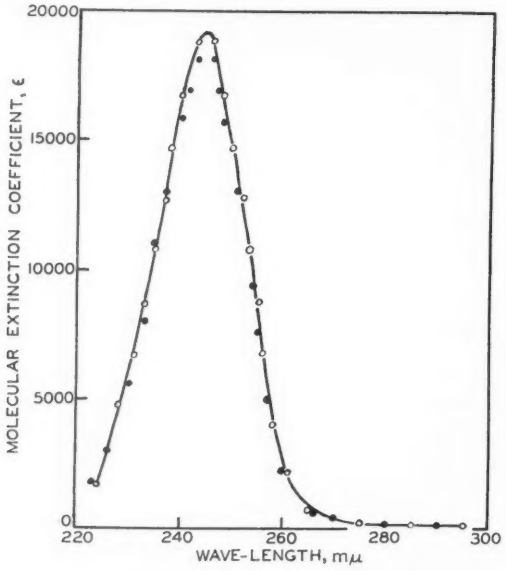


FIG. 1. *Ultra-violet absorption curves:* O, *natural product;* ●, *synthetic 2-mercapto-5,5-dimethyl-oxazoline.*

Experimental

Material

The seed was obtained from a commercial sample of grain screenings from a terminal elevator at Fort William, Ontario. After aspiration to remove chaff and straw, the mixture of seeds was separated into two fractions by immersion in ethylene dichloride. The density of this liquid is such that the oil-bearing

seeds float while the starchy seeds sink. The oil-bearing seeds were passed over a machine that separates seeds according to their shape. This device was constructed in the laboratory. The hare's ear mustard being of ovoid shape was thereby separated from the round and flat seeds that accompanied it. After a final cleaning in the laboratory aspirator, the seed contained less than 2% of impurities. The material was ground in a small roller mill and extracted with petroleum ether to remove fat.

Isolation of Sulphur-containing Substance

One hundred grams of fat-free crushed seed was stirred into 400 cc. of water at 37° C. The mixture was agitated on a shaking machine for four hours. The pasty mass was centrifuged in bottles and the recovered liquor was extracted repeatedly with chloroform, separation being effected with the aid of the centrifuge. The chloroform extracts were dried over potassium carbonate.

On evaporation of the solvent there remained 0.5 gm. of crystalline solid, m.p. 99 to 101° C. After recrystallization from dry ether it melted at 104 to 105° C. Further recrystallization from benzene, by cooling to 0° C., gave crystals melting at 108.5 to 109.5° C.

Analysis of the substance indicated the formula C_5H_9ONS, as shown in Table I.

TABLE I

ELEMENTARY ANALYSIS

Element	Calc'd. for C_5H_9ONS	Found
C	45.80	46.01, 46.04
H	6.88	6.98, 7.09
N	10.7	10.9 (Kjeldahl)
S	24.4	24.3
O	12.2	11.7 (By difference)

A molecular weight determination was inconclusive. Mol. wt. (Rast-Pregl.); found: 170,174. C_5H_9ONS requires: 131.

Structure and Properties

A sample of the substance melting at 108.5 to 109.5° was mixed with synthetic 2-mercapto-5,5-dimethyl-oxazoline, which melted at the same temperature. The mixture showed no depression in melting point. 2-Mercapto-5,5-dimethyl-oxazoline crystallizes in white, prismatic needles. The substance is tasteless to some persons, including the author, while others find it intensely bitter*. It is readily soluble in alcohols, chloroform, acetone, and ethyl acetate; moderately soluble in water, ether, and benzene; but insoluble in hexane. The solubility in benzene at 0° C. is about 4.0 gm. in 100 cc.

It does not combine with methyl iodide under ordinary conditions, nor does it form a picrate. When heated at 95° C. with sodium plumbite solution the mixture darkens gradually, and a precipitate of lead sulphide is visible after 30 min. It is oxidized rapidly by concentrated nitric acid.

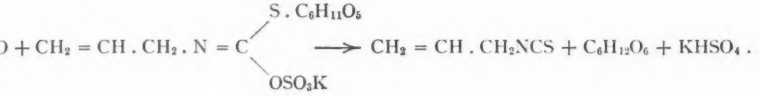
**References to taste throughout the text refer to the taste reaction of the author.*

Attempted Isolation of the Bitter Principle

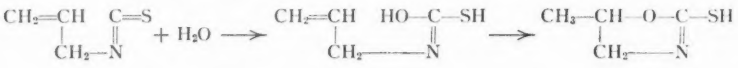
Quantities of the powdered, fat-free seed were extracted separately with methanol, 95% ethanol, dioxane, and ethyl acetate. In each case a bitter-tasting solid was recovered from the extract but none could be induced to crystallize. Methanol and ethanol were the best solvents, leaving the extracted residue devoid of bitterness. The bitter principle is insoluble in anhydrous acetone, ether, chloroform, and hexane.

Discussion

The sulphur-containing glycosides of Cruciferae species previously studied have yielded isothiocyanates of the general form RNCS. Thus sinigrin from *Brassica nigra* hydrolyzes as follows (2):

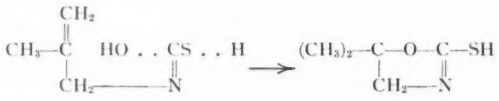


The production of a cyclic compound from such a mustard oil could be explained by assuming that water adds to the C=S linkage and that ring closure then takes place with migration of one H atom.



This change has never been observed during the hydrolysis of sinigrin. It is known, however, that the presence of a tertiary carbon atom at the double bond facilitates ring closure of aliphatic isothiocyanates and thioureas (1).

It is not unlikely, therefore, that the substance 2-mercapto-5, 5-dimethyl-oxazoline is formed from the isothiocyanate $CH_2{=}C(CH_3){-}CH_2{-}NCS$ by addition of water and ring closure—



Acknowledgments

Grateful acknowledgment is made to Dr. H. T. Clarke and Dr. H. Hirschmann, through whose kindness the absorption spectra determinations were carried out, and to Dr. H. A. Bruson, who supplied the synthetic material.

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THE PREPARATION AND SOME FLOW CHARACTERISTICS OF PLASTIC CASEIN[1]

By Wilfred Gallay[2] and James S. Tapp[2]

Abstract

The preparation of rennet casein has been studied on both a laboratory and plant scale, and the effect of the various factors in procedure on the quality of the casein produced is discussed. The details of preparation for a product of excellent quality are given. A simple heat resistance test showing the presence of impurities has been developed. The plasticity of a number of caseins has been measured by a direct method in a laboratory extruder, under pressure ranging up to 77,000 lb. per sq. in.; the maximum viscosity measured directly in this study was about one billion, seven hundred million poises. The flow-pressure curves obtained show no tendency towards a yield point, but show varying degrees of structural viscosity and may be fairly well represented by the relation $F = kP^n$, where F is the flow, P is the pressure, n and k are constants. Decrease in pH of the casein, decrease in ash, and increase in lactose content all lower the viscosity of plastic casein. Small increases in water content decrease the viscosity greatly. Up to 80° C. the temperature of drying has no appreciable effect on the viscosity. Moistening of casein plastic is discussed, as is also the distribution of water and vapor equilibria. Plastic casein is shown to exhibit streamline flow in extrusion, and plasticization is therefore more difficult to accomplish.

Plasticization of Casein

A casein plastic is prepared industrially by the homogenization of moistened casein granules and extrusion to the desired size, followed by either cutting directly to discs and hardening in formaldehyde, or by pressing the formed rods to sheets and hardening. The cured plastic is then machined to the desired size and form. The moistened casein is plasticized by means of a cylinder press fitted with a rotating screw which causes a rubbing and flowing of the casein over and into itself while transporting it forward to the extrusion orifice. The combination of the pressure and the heat developed brings about the desired homogenization. Facilities for cooling the casein during plasticization and gentle heating during extrusion are provided.

It is important to note that the speed of the screw and the clearances provided are fixed; the only variation possible in the operation of the extruder is the partial control of temperature afforded by the waterjacket around the screw. It is obvious therefore that the flow characteristics of the casein used must be such that within the limits of variation possible in the extruder plasticization will take place. If the viscosity of the casein is too low, the maximum rate of shear afforded by the extruder is insufficient to bring about a proper degree of plasticization, and the extruded material will consist merely of deformed casein particles adhering together in an irregular manner. If, on the other hand, the viscosity of the casein is too high, the rate of shear developed by the extruder produces excessive heating in the screw, and the material backs up through the feeding hopper as a result of the extremely high pressure developed.

[1] *Manuscript received August 11, 1938.*
Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada.

[2] *Chemist, National Research Laboratories, Ottawa.*

Casein for plastics manufacture must therefore possess a viscosity within the range of the extruder. Uniformity of quality is important so that a standard manufacturing procedure may be adopted and relied upon to yield the desired results.

The purpose of the present work was to study the factors affecting the flow of rennet casein and methods of preparation to yield a uniform product of high quality.

A laboratory extruder was used for the measurement of the viscosity of rennet casein under varying rates of shear, pressures up to about 77,000 lb. per sq. in. being employed. The flow-pressure curves obtained were regular, and this direct method of measurement is more accurate and permits a greater range of experiment than one other indirect method previously described (1).

Preparation

The preparation of rennet casein has been described by a number of authors, but, generally speaking, insufficient correlation has been made between the various factors entering into the preparation and the properties of the resultant casein, particularly with regard to its suitability for plastic manufacture.

Rennet casein is prepared by the addition of rennet to skim milk, followed by pressing and drying of the coagulated curd. The skim milk should be fresh, since a high pH allows the combination of inorganic salts with the casein; this results in a casein of greater viscosity and a consequently greater ease of plasticization in an extruder or press. The coagulated curd is gummy and the particles tend to grow to large lumps. These lumps are not only rubbery and very difficult to grind in a curd mill, but they also occlude lactose and lactalbumen which cannot be removed by washing. Shortly after coagulation and particularly when the curd suspension is heated, the coagulate begins to show marked syneresis with accompanied firming of the gel and loss of the sticky nature. This stickiness is probably due to albumen. It is obvious therefore that if the coagulate is not allowed to grow to larger lumps, but is kept in dispersed condition, then syneresis will occur with ready separation of water soluble constituents, which can then be removed by washing. It is to be noted that the particles formed by coagulation and syneresis retain their form through subsequent pressing and drying. If the particle size allowed is too small, however, the final grinding of the casein will produce a large proportion of fines. These fines have proved detrimental in the procedure now used in plastics manufacture, since on being moistened they occlude air and cake into lumps. Furthermore an appreciable proportion of the fines is lost during the separation of the casein curd from the whey by settling or screening.

It is therefore apparent that it is important to control the growth of the coagulate to optimum size. This may be effected by controlled agitation during coagulation and subsequent syneresis. The growth of the particles must be sufficiently slow so that time is available for this control. The amount of rennet used and the temperature for coagulation must be carefully chosen

so that a uniform means of control may be employed. Once the coagulate has grown to particles of a size greater than the optimum, powerful agitation is insufficient for dispersion. When syneresis is sufficiently advanced, the curd particles show no further tendency towards lumping. Syneresis is accelerated by slowly heating the curd suspension to about 60° C.

The water soluble impurities separated by syneresis may be removed by repeated washing of the curd. The washing efficiency is increased by the use of warm water; hot water causes an undue swelling of the curd. As much water as possible should be removed from the curd during pressing in order to shorten the time of drying. Prior to drying, the curd should be milled to shreds approximately twice the size desired in the final product; curd milling is necessary in order to avoid case hardening during drying. The drying temperature should be low with sufficient access of circulating air to ensure rapid removal of moisture.

These conditions for preparation were worked out in the laboratory, partly on a very small scale, but chiefly in batches of 80 lb. of skim milk. Additional experiments were carried out in casein plants on an industrial scale, from 4000 to 7000 lb. of skim milk being used in each experiment. In one plant engaged primarily in the production of acid caseins, no mechanical agitators were available. It was shown in several trials that it was impossible by hand-raking, even with four men working at a tank, to so control the particle size of the curd that large lumps could be avoided. In these experiments, the finished casein was of a very inferior quality. In a second plant, in which mechanical agitation was available but no variation in speed was possible, a very good product was obtained; the yield however was somewhat low owing to the production of fines which were lost in the removal of the whey.

The presence of lactose and albumen in the casein, if sufficient in amount, may cause a discoloration of the casein during drying even at a temperature of about 120° F. Smaller amounts, insufficient to discolor the casein powder, are readily detected however during the process of plasticization in the extruder. In the extruder a high temperature is attained for an appreciable period. The discoloration, particularly in an unpigmented plastic, is seen as dark streaks in the finished product. The effect of heating caseins to different temperatures for varying times was investigated, and a temperature of 150° C. for four hours was chosen as an arbitrary test for the heat resistance of the caseins. Under these conditions, available commercial caseins varied in color from ivory to deep brown. It was noted particularly that those caseins prepared in this study which had been carefully controlled during coagulation and therefore were well washed, showed excellent heat resistance. An obviously inferior grade of commercial casein was allowed to swell in water at 35° C., it was then thoroughly washed with warm water and dried at 50° C. The resultant casein had an appreciably increased heat resistance.

The effect of adding impurities artificially to a good casein was noted. Increasing amounts of lactose caused large increases in discoloration as a result of the heat test. Increasing amounts of albumen were much less

effective than lactose in this regard. Lactose alone was not affected by this heat treatment, and therefore it appeared that some combination of the protein and carbohydrate was responsible. Lactose treated with small amounts of acid and alkali and exposed to the heat test showed rapid darkening; therefore the effect may be due to the action of acidic or alkaline grouping in the protein on the sugar. The casein chosen for these experiments had been carefully prepared and washed, and had a pH of 6.9. Experiments showed that the lactose artificially added to the casein could be quite well removed by three washings with warm water.

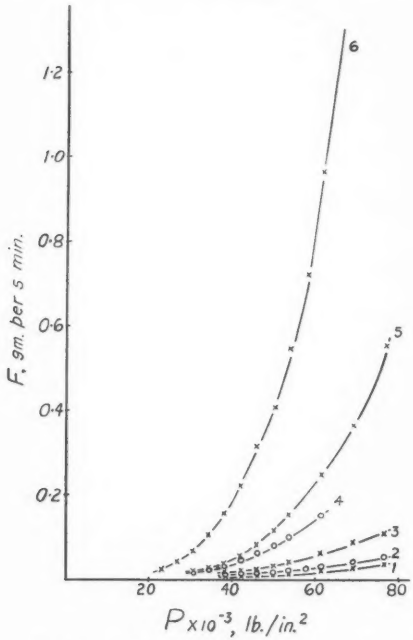

FIG. 1. *Effect of moisture content on flow of casein. Moisture contents—1, 27.10; 2, 27.35; 3, 28.00; 4, 28.30; 5, 28.55; 6, 29.25%.*

In many instances, spotty effects were obtained by the heat resistance test, the color of the granules varying from ivory to deep red or brown. The explanation of this lies in the fact that during the coagulation and syneresis in preparation, both large and small particles were obtained. The small particles were well washed, and they therefore discolored very little as compared with the larger lumps which occluded lactose and albumen and discolored badly in the test.

Caseins prepared in the laboratory and on a plant scale under the conditions noted for high quality showed excellent results when used in a commercial screw extruder for plastics manufacture.

*Effect of Moisture Content on Flow**

Casein swells in water and must be moistened prior to plasticization in order to provide sufficient medium for homogenization. Fig. 1 shows a series of flow-pressure curves for a rennet casein containing different amounts of water. The tremendous effect of small differences in moisture content is apparent. An increase of only 2.15% in moisture brings about an increase of 4700% in flow at a pressure of about 61,000 lb. per sq. in.

It is apparent from these results that the moisture content must be very carefully controlled. In commercial operation, the calculated amount of

* *All moisture contents have been calculated on the wet basis; e.g., 27% moisture content equals 38.89% moisture calculated on a bone-dry basis.*

water is added to the air-dry casein of known moisture content in an apparatus of the dough-mixer type. A reasonably good mixing is obtained and the amount of moisture lost by evaporation is small in comparison with the total mix. Such an apparatus is entirely inadequate for close control in laboratory work, and a closed roller mixer was developed for this purpose, by means of which in later experiments moisture contents could be so adjusted that the maximum error was less than 0.1%.

TABLE I

EFFECT OF MOISTURE CONTENT ON SPEED OF FLOW

Pressure, lb. per sq. in.	Flow (gm. per 5 min.) at moisture content of					
	27.10%	27.35%	28.00%	28.30%	28.55%	29.25%
23,060	–	–	–	–	–	0.0258
26,900	–	–	–	–	–	0.0415
30,740	–	–	–	0.0162	0.0169	0.0669
34,590	–	–	–	0.0209	0.0231	0.1046
38,430	0.0043	0.0093	0.0181	0.0305	0.0357	0.1555
42,270	0.0053	0.0099	0.0197	0.0440	0.0542	0.2204
46,120	0.0067	0.0117	0.0255	0.0598	0.0821	0.3165
49,960	0.0089	0.0158	0.0314	0.0788	0.1160	0.4075
53,800	0.0113	0.0188	0.0404	0.0970	0.1531	0.5490
57,650	–	0.0233	–	–	–	0.7195
61,490	0.0207	0.0301	0.0616	0.1510	0.2482	0.9670
65,330	–	–	–	–	–	1.3220
69,170	0.0265	0.0403	0.0878	–	0.3635	–
76,860	0.0338	0.0531	0.1080	–	0.5550	–

Casein granules vary greatly in size, and it was considered possible that the amount of moisture taken up varied with decreasing particle size. A sample of unscreened casein was moistened to 28% moisture content in the roller mixer according to the usual procedure. Then a separation into two fractions was made by inserting a cylindrical 30 mesh screen into the mixer and separating in the closed mixer. Determination of the moisture content of the −30 mesh, +30 mesh, and mixture of the two, however, showed no appreciable differences. In a further experiment equal amounts of +30 mesh casein with 10.30% water and −30 mesh casein with 0.45% water were mixed for the same length of time (15 hr.), and then moisture determination was made on the samples screened in the mixer. It was found that the +30 mesh casein then contained 7.35% moisture and the −30 mesh casein contained 3.95% moisture. The water content of the mixture was 5.70%. Casein therefore does not partake of the properties of an inelastic gel in the matter of equilibrium vapor pressure. It is important to note that a uniform mixture cannot be obtained by mixing two caseins of different moisture contents.

The heats of swelling of various caseins of different quality in water were measured, but the variations obtained were within the range of experimental error. Much more variation in results was obtained with varying degree of fineness of grinding of the caseins.

Effect of pH and Ash Content on Flow

The ash content of a rennet casein depends upon the pH of the milk from which it is prepared. Table II shows one series where the pH of the skim milk was allowed to fall by natural souring, and rennet caseins were prepared at intervals.

TABLE II

ASH CONTENT OF CASEINS FROM SKIM MILK AT VARYING pH

pH of skim milk	6.8 – 6.9*	6.2	5.9	4.6**
Ash content, %	8.9 – 9.1	7.76	5.74	0.56

* *Several samples.* ** *Natural sour.*

The viscosities of solutions of acid caseins increase with increase in ash content, and the particularly high viscosity of rennet casein solutions is to be ascribed chiefly to high content of calcium phosphate. It is to be expected therefore that the speed of flow of a rennet casein will increase with decreasing pH of the skim milk from which it was prepared.

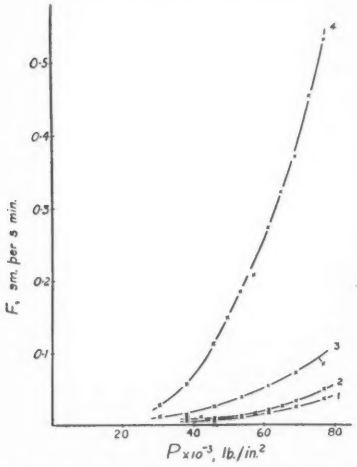

FIG. 2. *Effect of pH and ash content on flow of casein. Ash contents—1, 9.0; 2, 8.8; 3, 6.2; 4, 0.56%.*

The flow-pressure curves of four caseins differing appreciably in pH and in ash content owing to the pH of the milk are shown in Fig. 2. These four caseins after moistening and immediately prior to extrusion showed water contents of 27.20, 27.10, 27.15 and 27.15%.

Casein No. 4 of Fig. 2 was prepared from the same milk as the other caseins of this group but was allowed to reach the point of natural souring without rennet addition. This series shows the combined effect of ash content and pH of caseins on the speed of flow. All these caseins were coagulated to optimum particle size and thoroughly washed; the results showed that the pH of the caseins prepared from partly soured skim milk cannot be raised to a satisfactorily high value by washing. The heat resistance of these caseins showed a low lactose and albumen content.

Effect of pH on Flow

In order to separate the effects of pH and ash of caseins on the flow, the following series of experiments were carried out. A rennet casein was prepared from fresh skim milk; it showed an ash content of 8.7% and pH of 7.1.

Portions of this casein were then agitated at room temperature for several hours with hydrochloric acid solutions of different concentrations; the caseins were then filtered, pressed, and dried. The acid treatment had little effect on the ash. The pH values of the treated caseins were measured and the flow-pressure curves determined after moistening. Fig. 3 shows the results obtained. It will be noted that there is some variation in the moisture content of these caseins prior to extrusion, causing some change in the speed of flow. Inspection of the figures show, however, that the curves are undoubtedly in the correct order, and that the error caused by varying moisture is a minor one.

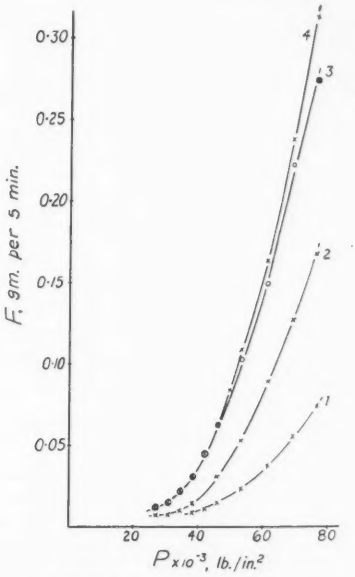

FIG. 3. *Effect of pH on flow of casein. pH values—1, 7.10; 2, 6.85; 3, 6.55, 4, 6.20. Moisture contents—1, 28.03; 2, 27.90; 3, 28.20; 4, 27.75%.*

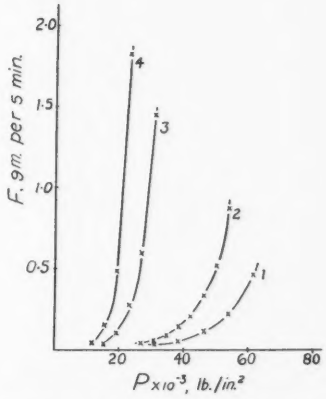

FIG. 4. *Effect of pH on flow of casein. pH values—1, 6.15; 2, 5.85; 3, 5.85; 4, 5.20%. Moisture contents—1, 25.55; 2, 25.63; 3, 28.35; 4, 28.10%.*

Further experimental data were available from another series in which portions of a commercial casein of inferior quality were treated with acid solutions of different concentrations, filtered, pressed, and dried. After moistening, the flow-pressure measurements were made. The results for two pairs of caseins, of different pH, each pair being at a different moisture level, are shown in Fig. 4. The marked increase in speed of flow with decreasing pH is well shown in this series also.

It is established therefore that a casein plastic with an absolute viscosity of some hundreds of millions of poises behaves as an ordinary lyophilic colloid with decreasing viscosity as the isoelectric point is approached.

Effect of Lactose on Flow

A rennet casein of excellent quality prepared in the laboratory and showing excellent heat resistance, high ash content and pH value, was treated at 65° C. for 20 min. with lactose solutions of varying concentrations. The caseins were then filtered, pressed, and dried. After moistening, flow measurements (Fig. 5) were made in the extruder. The variations in moisture content will account for the irregularities in the order with different pretreatments of lactose, but examination of the figures shows a definite increase in the speed of flow with increased lactose content.

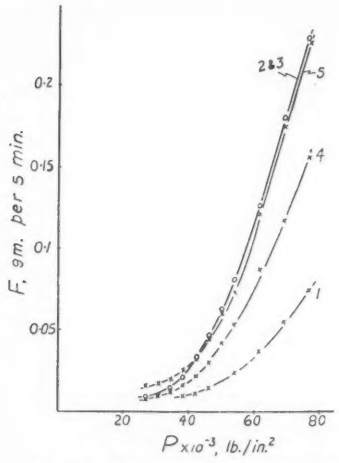

FIG. 5. *Effect of lactose on flow of casein. Lactose concentrations used in pretreatment—1, 0; 2, 0.4; 3, 1.5; 4, 3.0; 5, 6.0%. Moisture contents—1, 28.03; 2, 28.25; 3, 28.15; 4, 27.80; 5, 27.70%.*

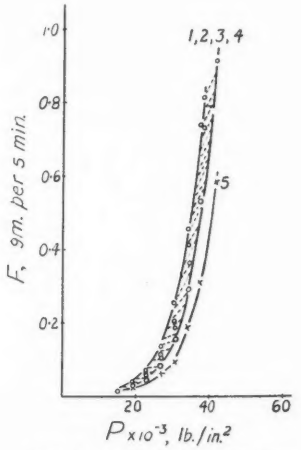

FIG. 6. *Effect of drying temperature on flow of casein. Drying temperature—1, 65°; 2, 50°; 3, 40°; 4, 40°; 5, 80° C. Moisture contents—1, 27.27; 2, 27.48; 3, 27.42; 4, 27.16; 5, 26.61%.*

Effect of Drying Temperature on Flow

Samples of a commercial casein were shaken for two hours with distilled water, then filtered, pressed, and dried for 24 hr. at temperatures ranging from 40° to 80° C. The caseins were then moistened, and the speed of flow measured in the extruder. Fig. 6 shows the results obtained.

It will be noted that there are no significant differences in flow; the casein dried at 80° C. is slightly more viscous than the others because of lower moisture content.

The flow-pressure curves are regular and show no tendency to a yield point on the pressure axis. The plastic casein exhibits structural viscosity, with the speed of flow increasing markedly with increased pressure; hence, the viscosity must be measured over a range of pressures in order that the plasticity may be characterized. The curves are of the exponential type

and are expressed fairly well by the equation $F = kP^n$, where F is the flow, P is the pressure, and k and n are constants. Fig. 7 shows some typical relations in logarithmic form.

Extent of Plasticization by Extrusion

Plasticization denotes a thorough homogenization of a material capable of plastic flow. The process is usually carried out by means of a combination of heat and pressure, and a variety of devices, depending on the properties of the material, are used to attain the plasticized state. In a rubber mill, tremendous pressures are attained in the nip between the rolls of the mill, and the rubber stock is rolled over and into itself and repeatedly subjected to the pressure. In a screw extruder, this action is attained to a much lesser degree, since different portions of a batch are not again mixed for further processing towards homogenization.

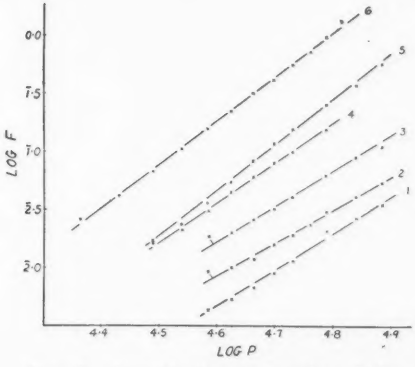

FIG. 7. *Logarithmic representation of flow-pressure curves. (See Fig. 1.)*

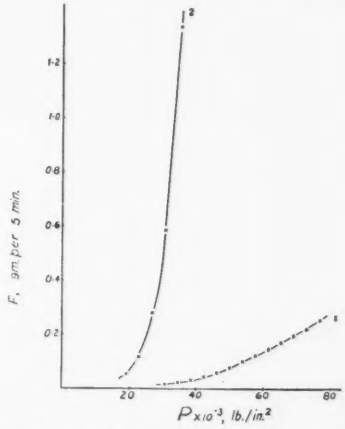

FIG. 8. *Effect of Baffle 1 on flow of casein. 1, with baffle; 2, without baffle. Moisture contents—1, 28.12; 2, 28.10%*

It was obvious that in the experimental extruder used in this study a much lesser degree of plasticization could be obtained than is possible in the commercial type of screw extruder, although the mode of action is somewhat the same. No attempt was made to introduce a rotating screw, owing to the great experimental difficulties involved in applying and measuring accurately the pressures, and in maintaining constant the nip between screw and cylinder wall.

In view of the foregoing, it was considered advisable to gain an idea of the extent of homogenization attained in the experimental extruder (shown in Fig. 9). A commercial casein was dyed blue and then samples of the undyed and dyed caseins were moistened separately to the same standard moisture content in the roller mixer. After subsequent thorough mixing of the two caseins for 15 hr. no appreciable transfer of color was noted. The mixture was then extruded and a thin cross section of the extruded thread examined

under the microscope. The result is shown somewhat diagrammatically in Fig. 11, *A*. It was noted that the result obtained was merely an irregular pattern of blue and undyed casein, due to the deformation of the particles while under pressure in the cylinder of the extruder and during passage through the orifice. It was noted further that the extruded thread was weak and deeply pitted around the circumference. It was then considered that insufficient plasticizing

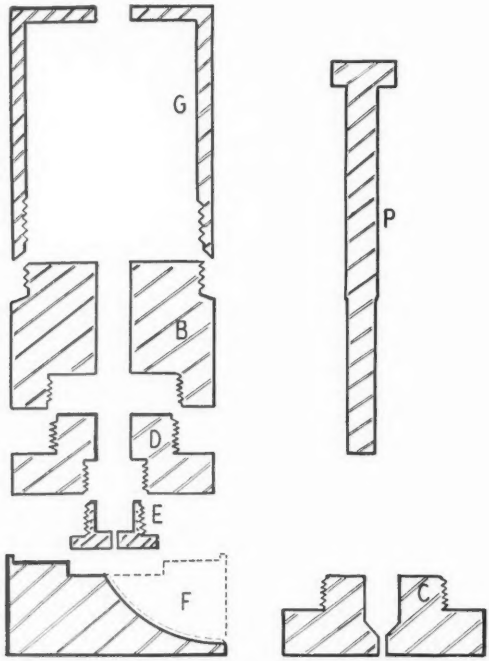

FIG. 9. *Laboratory extruder.*

force was being applied, and Baffle 1 (Fig. 10) was inserted into the cylinder of the extruder to provide a greatly increased rate of shear. The extrusion of the same mixture of dyed and undyed caseins described above was carried out with Baffle 1, and examination of the cross section of the thread obtained showed virtually no discontinuity of color. A cross section taken from the casein plug in the main bore of the cylinder showed by the lack of uniformity of color that very little plasticization occurred as a result of direct pressure alone. The extruded thread was much stronger than that obtained without the baffle and there was no appreciable pitting. Fig. 8 shows the flow-pressure curves obtained in these two experiments.

The effect of the baffle is well illustrated in these curves. At a pressure of 34,000 lb. per sq. in. the flow is reduced to about 1.7% of the value obtained under the same conditions without the baffle.

A second baffle was then constructed (Fig. 10, 2) in which the casein was forced through 10 regularly spaced holes prior to entering a collecting chamber. A casein that had been moistened and dyed by a plastics manufacturer was used for extrusion. The thread obtained was strong and the surface free

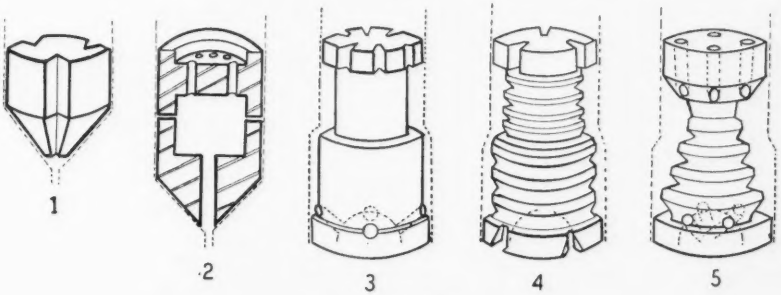

FIG. 10. *Baffles.*

FIG. 11. *Cross sections of extruded threads.*

of pitting. Examination of the cross section, however, showed 10 regularly spaced areas that differed so greatly in intensity of color from the remainder of the total area as to appear almost colorless. The cross section is shown somewhat diagrammatically in Fig. 11, *B*. It was obvious that the 10-hole pattern of the baffle was reproduced in the extruded thread after the casein had passed through the collecting chamber and extrusion orifice.

Baffles 3, 4 and 5 (Fig. 10) were then constructed in attempts to improve still further the plasticization of the casein. Each of these contained a four-hole pattern immediately above the collecting chamber. Extrusions were made, each of these baffles being used, and in all cases four regularly spaced areas were obtained of very low color intensity.

In discussion with Dr. D. F. Stedman of these laboratories, it was suggested by him that the flow of rennet casein through such an extruder would

be of a streamline character and that non-uniform portions of the unplasticized casein might be separated during flow in this way. The following experiments were then carried out in order that the possibilities of streamline flow might be observed. Two samples of a commercial casein were dyed red and blue, and the two caseins separately moistened to the same standard water content. After insertion of Baffle 5 and assembly of the extruder, a strip of pasteboard of width equal to the diameter of the cylinder bore was inserted into the bore; this gave two separated portions. These were filled with the red and blue caseins respectively and the pasteboard was carefully removed. The extrusion was then carried out, and the cross section of the extruded thread examined under the microscope. The cross section is represented in Fig. 11*C*, The four nearly colorless areas were present as usual owing to the baffle, and the total area was quite sharply and distinctly divided into red and blue halves. To ordinary visual examination, also, the thread appeared to consist of two hemi-cylinders, one red and one blue, which had been caused to adhere. The experiment was repeated with the same caseins, Baffle 3 being used in place of No. 5. The result is shown in Fig. 11*D*. The result was virtually the same except that some of the blue casein had been displaced, probably during the filling of the cylinder.

Examination of dyed casein granules showed that most of the dye had been taken up by the outer portion of the granules, and that the intensity of color falls off quite sharply towards the interior. Attempts were made to ascertain the distribution of water in the granules after moistening, but no definite results were obtained. It would appear however that the intensity patterns shown in Fig. 11, *B*, *C*, and *D*, must be due to a separation of the dyed and undyed portions of the granules by virtue of their different susceptibility to plasticization under the applied pressure. The exterior dyed portion is apparently plasticized to a greater extent than the remainder, and the latter portion is forced through the holes, surrounded by the more plasticized material which flows more readily under the rate of shear brought about by the holes. Streamline flow of the separate threads then takes place through the mass of casein in the collecting chamber and the threads emerge through the final orifice and show the four-hole pattern as areas of low color intensity. It is quite possible that the distribution of water in the moistened granule is such that more ready plasticization of the outer portion of the granule takes place. This effect may act in addition to the greater shear suffered by this portion. It may be concluded from the appearance of such patterns that plasticization in such an extruder is far from complete.

Experimental

Preparation

In some instances three litre batches of skim milk were processed in four-litre beakers set in constant temperature baths. Most of the experimental runs were carried out, however, with 80 lb. batches of skim milk. The coagulation vessel was a cylindrical steam jacketed, metal tank, of about 12 gal. capacity, rounded at the bottom and fitted with a gate-valve at the bottom for drainage.

The inside was coated with a corrosion resistant lacquer. An automatic temperature regulator, by means of which the temperature could be controlled if necessary to $\pm 1°$ F., was fitted on the entrant steam line. The agitator was attached to the rim of the tank, and since the speed of the motor used was not adjustable, agitation was controlled by hand operation of an on-off switch. The curd, contained in cloth bags, was pressed between the plates of a multiple-leaf, laboratory model, horizontal filter press. The curd was ground in a small hand-driven disc mill and the casein was dried in thin layers on trays by means of air from a fan heated by a specially constructed electrically heated coil placed vertically in front of the fan. It was noted that the use of gas burners for this purpose resulted in a discoloration of the caseins. A roller mill such as that used for the grinding of grain was found to be excellently suited to the final grinding of casein, with good throughput and little production of fines. A large number of experimental batches were made, such factors as the temperature of coagulation, pH of the skim milk, amount of rennet, time interval between visible coagulation and heating of the curd, the number of washes and the temperature of the wash water, extent of curd grinding, drying temperature, and others being varied.

The procedure developed for a rennet casein of an excellent quality was as follows: 80 lb. of fresh skim milk was placed in the coagulation vessel and the temperature raised to 38° C. with continuous agitation. Then 250 cc. of Hansen's Standard Rennet which had been diluted 40 : 1 was added. This is equivalent to 2.8 oz. per 1000 lb. of skim milk. After two minutes agitation, the stirrer was stopped. Visible coagulation was noted in about 18 min. and the curd particles were allowed to grow to about 3 to 4 mm. diameter, the size being controlled by the extent of agitation. Five minutes after visible coagulation, the mixture was slowly heated to 55° C. over a period of 20 min. with constant agitation after a temperature of 45° C. was reached. Then the suspension was drained into a washing vessel, the curd was allowed to settle and the whey poured off. The curd was washed three times with water at 90 to 100° F., with three minutes' agitation each time in the coagulation vessel followed by drainage and thorough removal of the wash water. The final wash water was colorless and clear and the curd consisted of small discrete granules that had no tendency to adhere. The curd was then pressed for one hour and ground to particles 2 to 3 mm. in diameter. The curd contains about 60% moisture at this stage. Drying was carried out at about 45° C. with good circulation of air. The dried casein was again ground, it being necessary only to separate the granules formed by the grinding of the pressed curd. The final product consisted of white granules appearing translucent and sometimes transparent under magnification. The ash content was about 9%, the pH 6.9 to 7.0, and the heat resistance excellent. Preparations on a plant scale were carried out in a similar manner, except that the curd was washed either in the coagulation tank or in a movable tank fitted with filtering duck. Direct steam was used for heating the milk in 4000 to 7000 lb. batches. Pressing and curd milling were carried out in the usual commercial

apparatus, and drying was effected in a tunnel drier fitted with arrangements for a partial recirculation of air.

Analysis

Moistures were determined by heating to 105° C. for three hours. All pH measurements were made electrometrically, a glass electrode being used. Ash determinations were made by direct calcination in a muffle after charring at a low heat. Check determinations with added soluble calcium salts showed that sufficient calcium was present in the rennet casein to retain all the phosphorus.

Moistening Prior to Extrusion

It was found necessary for close control to moisten the casein in a closed vessel. The moist casein adheres to the walls of the mixing vessel, and ordinary shaking does not bring about the proper distribution of water. The method finally developed was as follows: 30 to 50 gm. of air-dry casein was placed in a cylindrical screw-top bottle of about 200 cc. capacity together with several glass alleys, and the calculated amount of water added. The total diameter of the glass spheres was somewhat greater than the height of the bottle. After a brief shaking by hand, the bottle was placed on its side on a rolling device, and the contents was thoroughly mixed by rolling, usually for an overnight period. There was virtually no sticking of the casein to the container.

Extrusion

1. *Apparatus*

The extrusion apparatus, Fig. 9, consisted essentially of a hardened and polished steel piston *P* and a corresponding cylinder with a small orifice at one end. The cylinder used in earlier experiments consisted of two threaded sections, *B* and *C*, which, after assembly, rested on the steel block *F*. In this position, the orifice opened into the milled slot (9 mm. wide) in *F*. Later, section *C* of the cylinder was divided into two parts, *D* and *E*, to facilitate the introduction and removal of baffles.

The piston guide *G* was turned from steel shafting. It had a wall thickness of 5 mm., an over-all diameter of 71.5 mm. and a height of 81.5 mm. The main cylinder, *B*, was 72.5 mm. in diameter, 51.5 mm. in height, and had a bore diameter of 10.345 mm. Cylinder section *D* had a height of 28.5 mm. and was otherwise of the same dimensions as *B*. The large bore of section *E* was 12 mm. and the extrusion orifice was 1 mm. in diameter. The piston *P* was 134.5 mm. in total length and had three different diameters as follows:—(*a*) the diameter of the section extending 74 mm. below the head was 11.090 mm., (*b*) that of the next section, 31 mm. long, was 10.285 mm., and (*c*) that of the remaining section, 20 mm. long, was 10.330 mm.

Baffle 1 was turned roughly from a piece of brass rod; it had an over-all height of 13.5 mm. and its other dimensions were such as would enable it to

fit snugly into the tapered inner bore of cylinder section *C*. Three grooves, $\frac{1}{16}$ in. deep, were cut on its outer surface. Baffle 2 was made in two parts from cold rolled steel shafting. The over-all length was 20.5 mm. The upper section had 10 holes, 1 mm. diam., drilled as indicated in Fig. 10. The lower part had one central hole 1.56 mm. in diameter. The entire unit fitted closely within the bore of section *C*. This baffle was used only once, as it expanded under operation and became jammed in the cylinder. It was for this reason that *C* was drilled out and replaced by *D* and *E*.

Baffles 3, 4, and 5 were each about 22 mm. in height. They fitted closely at top and bottom into sections *D* and *E* respectively. This arrangement ensured ready removal of the baffle from either *D* or *E* after an experiment. The dotted lines in Fig. 10 represent the cylinder bore and indicate the clearance at points along the baffle. Baffle 3 had 11 grooves at the top and four holes of 1.17 mm. diam. at the bottom leading to the small collecting chamber immediately above the orifice. In Baffle 4, there were three grooves at the top and three grooves at the bottom. Baffle 5 had four holes of 1.17 mm. diam. at the top and four holes of the same diameter at the bottom above the collecting chamber.

2. Procedure

Sections *B* and *D* were put together and the piston, lightly oiled, was worked back and forth in the bore two or three times, and was then wiped free of oil with a clean rag. Section *E*, together with the baffle to be used, was screwed into place. The bore was filled quickly with the moistened casein, the piston was inserted and pressed down by hand, then the piston was removed and more casein added and pressed down until the final level of casein was about $\frac{1}{4}$ in. from the open end of the cylinder. The guide *G* was set in place and the piston inserted. The assembled apparatus was set on the block *F* and the whole placed between the plattens of an Amsler hydraulic press. A $\frac{1}{4}$ in. disc of aluminium was placed between the head of the piston and the upper platten in order to equalize the pressure over the entire area of the head. The pressure was slowly increased to a high value and about 1 ft. of thread allowed to extrude before the pressure was dropped and actual measurements of flow were begun. The pressure was set at the lowest desired level and after several minutes the emerging thread was severed flush with the orifice by means of a specially constructed chisel, and at the same time a stop watch was started. The pressure was held steady without difficulty by manual control, the maximum deviation being ± 50 lb. gauge (about 380 lb. per sq. in.). After the desired time interval, the thread was cut off flush with the orifice and immediately transferred to a screw-top bottle. Samples were obtained in a similar manner at all the required pressures. The samples were weighed and the weight for five minutes extrusion used in plotting the flow-pressure curves. Extrusion was not continued in any experiment when the bottom of the piston came within $\frac{1}{4}$ in. of the top of the baffle. After a complete extrusion experiment under a range of pressure, the whole unit was

warmed with hot water or steam, the head of the piston was held in a vise, and the cylinder was pulled away with a twisting motion. There was no jamming of the piston, and it was found that no casein crept past the advancing piston during extrusion.

Acknowledgment

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THE DETERMINATION OF TRYPTOPHANE BY A MODIFIED GLYOXYLIC ACID METHOD EMPLOYING PHOTOELECTRIC COLORIMETRY[1]

By J. L. D. Shaw[2] and W. D. McFarlane[3]

Abstract

An accurate and reliable method for the estimation of tryptophane is described. It is based on the glyoxylic acid reaction, and involves the use of the Evelyn photoelectric colorimeter. The technique makes it possible to ascertain readily whether the color being measured is due only to tryptophane.

The method has been applied to casein, of which, if necessary, only 25 mg. is required. The tryptophane determination is readily accomplished on a solution obtained by dissolving the casein in 10 or 20% sodium hydroxide or 5% formic acid by heating for a few minutes. With respect to alkali hydrolysis of casein under pressure, tryptophane is unstable in the sodium hydroxide hydrolysis, but is very stable in the baryta hydrolysis. The age and source of the casein are shown to be factors causing variations in the tryptophane content of different samples of casein, a variability which has been observed by a few previous workers.

Introduction

A thorough perusal of the literature reveals that although many methods have been proposed for the estimation of tryptophane there still remain unexplained a number of anomalies and discrepancies in the results. Lack of specificity of the reagent used in the colorimetric methods and failure to obtain a suitable standard for comparison have been the main reasons for questioning the results obtained by means of these methods. Before absolute confidence can be placed in a colorimetric method it must be shown that the color measured is due to tryptophane and to tryptophane alone. It is virtually impossible to demonstrate this by the ordinary methods of colorimetry, but, as will be shown later, this point can be checked when a photoelectric colorimeter is used.

Alkali hydrolysis is still widely used in the determination of the tryptophane content of proteins, although several workers have stated that tryptophane is unstable during this treatment. By use of the methods to be described in this paper, a critical study has been made of the determination of tryptophane in sodium hydroxide and barium hydroxide hydrolyzates of casein. Casein is the protein to which the methods of tryptophane estimation have been applied most frequently. Some 25 different values, ranging from 0.5 to 2.2%, for the tryptophane content of casein are given in the literature. However, as a few workers have noticed, different samples of casein may give different values for tryptophane when analyzed by the same method. Too frequently, nothing has been said about the source, mode of preparation, or the purity of the casein used. In no case has mention been made of the moisture content of the casein. As indicated later, this is variable and con-

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siderable, and should be taken into account in the expression of the results of tryptophane determinations.

When the importance of tryptophane in animal and plant metabolism is considered, it is very surprising that these needs have not yet been met. This paper presents the results of what is believed to be a successful attempt to fulfil these needs in the estimation of tryptophane in proteins.

Experimental

I. Development of an Improved Method

The tryptophane used was the *l*-form*, and its purity was established by determining its total nitrogen content by means of the micro-Kjeldahl method, the digestion mixture of Campbell and Hanna (1) being used. Digesting for one hour or longer with this mixture gave results which were constant and of the theoretical value. Accurate results were not obtained with the following digestion mixtures:— (i) copper sulphate, potassium sulphate, and concentrated sulphuric acid; (ii) mercuric oxide, selenium oxide, and concentrated sulphuric acid; and (iii) precipitated red selenium and concentrated sulphuric acid.

The colorimeter used was of the single cell photoelectric type, designed by Evelyn (3); it was found to be admirably suited to this study.

Twelve published methods for the estimation of tryptophane were thoroughly examined and, for various reasons, discarded. We believe that the method offering the best possibilities was Winkler's (9) adaptation of the Hopkins-Cole reaction. Her method was carefully checked in every detail and modified in the following points:—

(1) The glyoxylic acid solution was prepared according to the method of Pesez (6), as follows: To 100 ml. of a 5% aqueous solution of oxalic acid in a 150 ml. beaker was added 3.0 ml. of a $N/5$ mercuric chloride solution and a few small pieces of aluminium wire. The beaker, covered with a watch glass, was placed on a boiling water bath and heated for five minutes after the first appearance of bubbles on the aluminium wire. After the mixture had been allowed to stand for a further five minutes at room temperature, it was filtered, and 2.0 ml. of concentrated sulphuric acid was added to the filtrate. The solution was stored in the ice box to be used as required. Prepared in this way, the glyoxylic acid was stable for two weeks or longer.

(2) Winkler placed particular stress on the importance of intense cooling during the addition of the sulphuric acid in the reaction. The writers found this to be unnecessary.

(3) All conditions and concentrations used in the reaction were examined to obtain maximum color development, *i.e.*, *(a) Strength of sulphuric acid*—the concentrated acid (sp. gr. 1.84) was found to be the only suitable strength; *(b) amount of concentrated sulphuric acid*—5 ml. proved to be the optimal amount (Fig. 1, *a*); *(c) time of standing between the addition of the concentrated*

* *Obtained from the Eastman Kodak Co., Ltd.*

acid and heating in boiling water—the length of time appeared immaterial, at least over the periods tested, *i.e.*, up to 20 min.; *(d) time of heating in boiling water*—five minutes was found satisfactory for the maximum development of the color (Fig. 2); *(e) amount of glyoxylic acid*—0.50 ml. of the glyoxylic acid solution was found to be the optimal amount (Fig. 1, *b*); *(f) amount of copper*

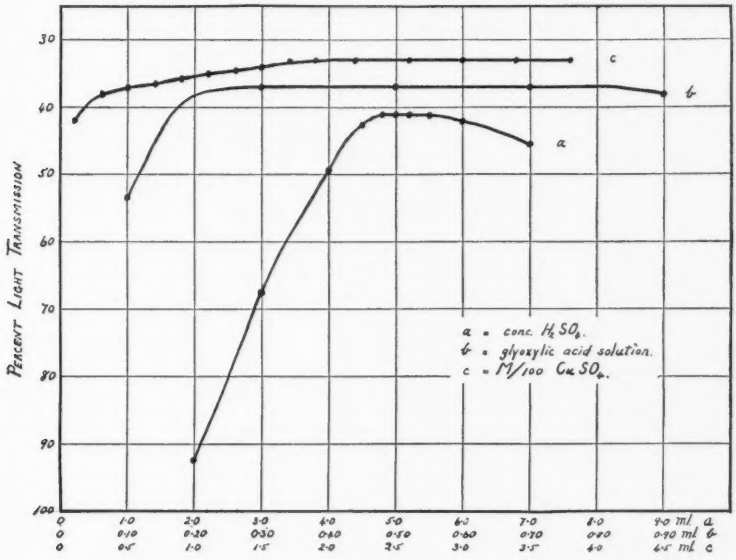

FIG. 1. *Effect of (a) concentrated sulphuric acid, (b) glyoxylic acid, and (c) copper sulphate on the color intensity. (Light filter = 540 mμ.)*

sulphate—0.50 ml. of a *M*/25 solution appeared to be perfectly satisfactory (Fig. 1, *c*); *(g) stability of the color*—found to be stable for at least three hours; *(h) reproducibility*—in this connection, it was ascertained that there was no necessity to standardize the glyoxylic acid, provided that it was prepared in the manner described above.

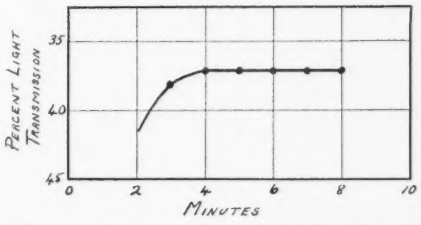

FIG. 2. *Effect of the length of time of heating on the color intensity. (Light filter = 540 mμ.)*

From the results obtained in the above-mentioned tests, the method finally adopted was as follows:—

From 0.10 to 2.00 ml. of an aqueous solution, containing 0.005 to 0.150 mg. of tryptophane, was placed in a 10 ml. glass-stoppered measuring cylinder, and 0.50 ml. of the glyoxylic acid solution and 0.50 ml. of a *M*/25 copper

sulphate solution were added. The volume was made up to 3.00 ml. with water. A total volume of 5.0 ml. of concentrated sulphuric acid was added from a burette in portions of 0.5, 1.0, 1.5, and 2.0 ml., the cylinder being well shaken under running cold tap water after the addition of each portion. After the addition of the acid, the cylinder was allowed to stand for about ten minutes in cold tap water and then placed in boiling water for five minutes. After the solution had been allowed to cool to room temperature, it was diluted to 10 ml. with a 5 : 3 (by volume) concentrated-sulphuric-acid–water mixture; this did not result in any heating. The solution was finally transferred to a clean, dry colorimeter tube. The reading was made after 15 min. The blank used was a colorless solution containing tryptophane in equal volume and concentration to the test solution and treated in the same way as the test solution, but to which no glyoxylic acid solution was added.

To prepare the calibration curves, 15 different amounts of a tryptophane solution were used, being equivalent in range to 0.007 to 0.141 mg. of tryptophane. Each solution was tested in duplicate in the manner described above. Two sets of readings were taken, a 540μ light filter being used, followed by a 520μ light filter; two calibration curves were thereby obtained. The choice of these two light filters was based on the absorption spectrum of the blue-violet color (Fig. 3). These two curves served, in the

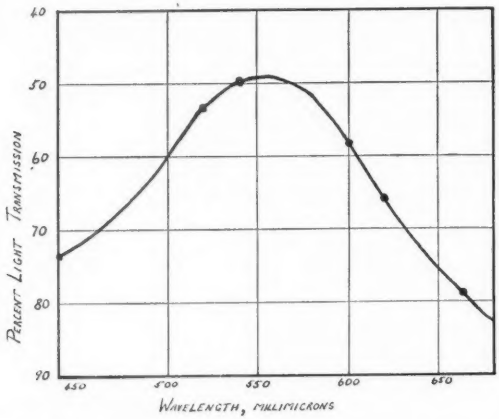

FIG. 3. *Absorption curve of color produced in the glyoxylic acid reaction.*

later work, to prove whether the color measured was due to tryptophane alone. With the exception of a few points where the highest concentrations of tryptophane were employed, the calibration curves obey Beer's law (Fig. 4), thus proving that the conditions and concentrations employed are optimal for the development of the color.

II. Application of the Method to the Determination of the Tryptophane Content of Casein

Casein was prepared from the winter milk of a herd of stall fed cows. The skim milk was used and the casein precipitated by the method of Moir (5) and purified according to the directions of Van Slyke and Baker (8, pp. 10, 11). The moisture content was determined by the technique of Snyder and Hansen (7) and found to be 8.41%. All results given below are expressed on the basis of the air-dried material, unless otherwise stated.

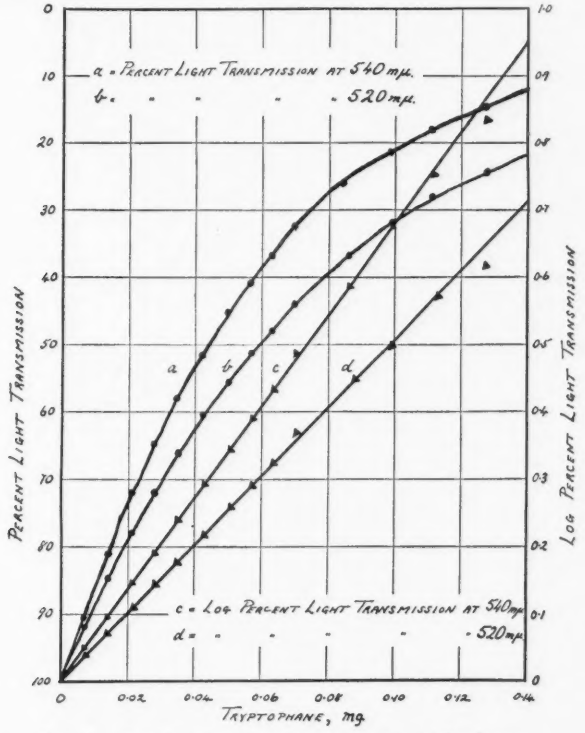

FIG. 4. *Calibration curves and application of Beer's law.*

One gram of the casein was dissolved in 20 ml. of a 20% solution of sodium hydroxide by heating on the boiling water bath for several minutes until a clear solution was obtained. The solution was transferred to a 200 ml. volumetric flask and made up to volume with water. Duplicate determinations of tryptophane in 1.00 ml. aliquots were made by the procedure described above. The blank also contained 1.00 ml. of the solution and was treated in exactly the same manner, but no glyoxylic acid solution was added. The readings with the two light filters corresponded to 1.16% of tryptophane. The deter-

mination was repeated several times and was also carried out with 20 ml. of a 10% solution of sodium hydroxide, or 20 ml. of a 5% formic acid solution, as the solvent for the casein. The same result was obtained in all determinations.

Although there was, most likely, a certain degree of hydrolysis, at least in the determinations in which sodium hydroxide was used as the solvent, it is doubtful whether the tryptophane was actually liberated from the protein combination. Hence, this method of examining the tryptophane content of casein will be referred to as simple solution in contradistinction to the far more drastic treatment of alkali hydrolysis under pressure.

III. The Stability of Tryptophane during Alkali Hydrolysis under Pressure

In the investigation of sodium hydroxide hydrolysis under pressure, 1 gm. of the casein was hydrolyzed with 20 ml. of a 20% solution of sodium hydroxide in the autoclave as recommended by von Deseö (2). The hydrolyzate was treated with sulphuric acid and kaolin as outlined by Folin and Marenzi (4). A new Pyrex Erlenmeyer flask was used for each hydrolysis, and it was also ascertained that no tryptophane was absorbed on the kaolin. Comparison of the results with those obtained as described above by simple solution of the casein shows that there was always a loss of about 10% of tryptophane under different conditions of temperature, pressure, and time of hydrolysis when a 10, 20, or 30% solution of sodium hydroxide was employed.

To examine baryta hydrolysis under pressure, 1 gm. portions of the same casein were hydrolyzed in the autoclave with 20-ml. aliquots of saturated baryta solution for different lengths of time (up to five hours), at different temperatures (120° and 150° C.), and pressures (2.0 and 4.5 atm.). The hydrolyzate in each case was immediately acidified with 20 ml. of 7*N* sulphuric acid and rapidly cooled. The barium sulphate was filtered off and thoroughly washed with water. The filtrate and washings were made up to 200 ml. with water, and 1.00-ml. aliquots were taken for the determination and for the blank.

The resultant color indicated about 1.3% of tryptophane in every case, but the disproportionality in the readings with the two light-filters showed that the color was not identical with that given by pure tryptophane. However, when the hydrolyzate was first extracted three times with ether, and the ether dissolved in the hydrolyzate was removed by evaporation *in vacuo* at room temperature, the determinations on 1.00-ml. aliquots of the solution gave the pure tryptophane color and corresponded in every case to 1.16% of tryptophane. The results, therefore, were in excellent agreement with those obtained when the casein had simply been dissolved in sodium hydroxide or formic acid. Obviously, baryta hydrolysis under pressure does not result in any loss of tryptophane.

The nature of the substance or substances removed by the ether extraction, and giving a color with glyoxylic acid, was not investigated. The results indicate, however, that the interfering substance, which may be indole or skatole, was not formed from tryptophane during hydrolysis in the autoclave.

IV. The Variation in the Tryptophane Content of Different Samples of Casein

Two other samples of casein, both several years old, were available. These had moisture contents of 10.08 and 7.80% respectively. In the determination of the tryptophane content by dissolving in a 5% solution of formic acid as described above, these samples gave less than 1% of tryptophane. Evidently, the age of the sample affects the tryptophane content of casein.

A fresh sample of casein was prepared from the milk of the same herd of cows, but after they had been on pasture for two months. The casein was obtained in exactly the same manner as before; it contained 10.87% of moisture. Repeated direct determinations of the tryptophane content of a sodium hydroxide solution of this sample of casein gave 1.19%.

The tryptophane content of the various samples of casein are given in Table I.

TABLE I

A COMPARISON OF THE TRYPTOPHANE CONTENT OF VARIOUS SAMPLES OF CASEIN

Sample	Age	Moisture content, %	Tryptophane, gm. per 100 gm. moisture-free casein			
			Solution		Hydrolysis	
			NaOH, 10 or 20%	H·COOH 5%	NaOH, 20%	$Ba(OH)_2$, sat'd
Authors'—						
Sample from summer milk	Fresh	10.87	1.33	—	—	—
Sample from winter milk	Fresh	8.41	1.25	1.25	1.04	1.25
British Drug Houses "Light White Soluble"	8 years	10.08	—	1.09	—	—
Kahlbaum Co.	25 years	7.80	—	0.92	—	—

V. Amount of Protein Required for the Tryptophane Estimation

Although the above-mentioned tests were carried out with 1-gm. portions of the air-dried sample, it was evident that as small an amount as 25 mg. would be sufficient. On dissolving 25 mg. of casein in 1 ml. of a 10% solution of sodium hydroxide with the aid of heat, making up to 5.00 ml. with water, and using 1.00 ml. for the tryptophane determination, results were obtained which were comparable in accuracy and reproducibility with those of the tests in which larger amounts of the protein were used.

Note Added October 7

In making some tryptophane determinations recently we observed that when certain samples of concentrated sulphuric acid (c.p. grade) were employed, a yellowish-brown color developed in the blank determination. This leads to erroneous results even in determinations on aqueous solutions of pure tryptophane. The difficulty was overcome by distilling the acid.

Acknowledgment

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TANNINS AND NON-TANNINS OF THE BARKS OF SOME EASTERN CANADIAN CONIFERS, PARTICULARLY WHITE SPRUCE[1]

By W. E. Graham[2] and A. Rose[3]

Abstract

The tannin content of the barks of several Eastern Canadian conifers has been determined by standard methods in an attempt to assess their value as sources of tanning extracts.

A more intensive study has been made of the extractable materials of white spruce bark. The chemical reactions of the extract from this bark show that the tannins therein are typical examples of the catechol or condensed group. Comparison of the ratio of tannin to non-tannins and the buffer index of this extract with the corresponding values for several commercial extracts indicates that the spruce extract would probably be fairly astringent. The titration curves of various fractions of the extract show some indications of the character of the non-tannin constituents.

The available literature on the subject of natural sources of tannin has been found to contain little or no quantitative information regarding the tannin content of the barks of the more abundant conifers of eastern and central Canada, with the exception, of course, of the well known Eastern hemlock *(Tsuga canadensis)*.

Large quantities of bark from the conifers cut for pulpwood in Eastern Canada (particularly white spruce, black spruce, and balsam) are wasted or destroyed annually. These barks, among others, have been examined in these laboratories during recent years as potential sources of tanning extracts.

A. General Analytical Results

Analytical Methods

The air-dried samples were ground in a Wiley mill. Extractions were carried out (i) according to the methods of the American Leather Chemists' Association (A.L.C.A.), suitably modified Pyrex Soxhlet extractors (43 mm. diameter) being used, or (ii) in some cases, according to the methods of the International Society of Leather Trades' Chemists (I.S.L.T.C.), a modified Koch extractor being used. In the first method the filtering medium used in the extractors was glass wool, washed with dilute hydrochloric acid, then with distilled water. The extracts were analyzed according to the respective official methods based on the two modes of extraction, rapid cooling being used in the A.L.C.A. method.

It is of interest to note at this point that the bark of the balsam *(Abies balsamea)*, which is the source of the Canada balsam of commerce, could not be extracted with hot water, even after removal of resinous material by means of ethyl ether, because of the presence of pectinous matter. Since balsam,

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black spruce, and white spruce stands are closely intermingled in the principal pulpwood regions of Ontario and Quebec, this fact is of some importance in appraising the possibilities of one or all of these barks as sources of tanning extracts.

TABLE I
ANALYSIS OF BARKS

Bark of	Sample No.	Source	Method of extraction	Soluble solids, %	Soluble non-tannins, %	Tannin, %	Purity, %
Eastern cedar (*Thuja occidentalis* Linn.)	1	Ont.	A.L.C.A.	10.6	7.6	3.0	28
Red spruce (*Picea rubra*)	1	N.B.	A.L.C.A.	21.40	9.18	12.22	57.1
Black spruce (*Picea mariana*)	1	Ont.	A.L.C.A.	27.70	15.54	12.16	43.8
	2	N.B.	A.L.C.A.	24.00	13.97	10.03	41.7
	3	N.B.	A.L.C.A.	23.85	12.92	10.93	45.8
	4	N.B.	A.L.C.A.	21.48	11.97	9.51	44.2
	5	Ont.	A.L.C.A.	22.03	13.71	8.32	37.7
White spruce (*Picea canadensis*)	1	Que.	A.L.C.A.	40.5	18.5	22.0	54.3
	1	Que.	I.S.L.T.C.	32.3	11.5	20.8	64.4
	2	Que.	I.S.L.T.C.	33.0	12.8	20.2	61.2
	3	Que.	A.L.C.A.	42.2	21.1	21.1	50.0
	3	Que.	I.S.L.T.C.	32.3	13.0	19.3	59.6
	4	Ont.	A.L.C.A.	37.6	15.3	22.3	59.3

Analyses of the barks of three species of spruce and one of cedar are given in Table I. All results are reported on the moisture-free basis. "Purity" of the extract is defined as:

$$\frac{\text{Tannin}}{\text{Soluble solids}} \times 100\%.$$

B. White Spruce Bark: A Preliminary Investigation

The consistently high tannin content of bush-peeled white spruce bark, as shown in Table I, makes it a possible source of concentrated tanning extracts, provided that the economic obstacles involved in stripping, drying, sorting, and transporting the bark are not insuperable, and that the physico-chemical properties (color, purity, astringency, degree of fixation by hide fibre) of the extracts prepared from it are satisfactory. It is recognized that separation of white spruce bark from black spruce and balsam barks, either in the bush or at the point of shipment, might not be economically feasible even if practically possible. The tannins of all the eastern spruces are probably very similar chemically, and since white spruce bark is the most convenient of the three as a laboratory source of moderately concentrated extracts, it was selected for this exploratory study.

Only large-scale tests in the tannery are of real value in the assessment of the merits of a new extract; there are several factors, particularly in heavy leather manufacture, which are difficult to duplicate in the laboratory.

A study of some of the simpler chemical tests has been included in the present investigation for purposes of classification, more particularly because some Canadian tanners have expressed the opinion that the spruce barks contain a "pseudo-tannin" as opposed to a "true tannin". The formation of a precipitate on addition of bromine water, and also on refluxing with formaldehyde in hydrochloric acid solution, is a characteristic reaction of tannins of the catechol or condensed type. The "formaldehyde precipitation figure" of a tan liquor is the weight of the precipitate obtained on addition of formaldehyde expressed as a percentage of the weight of the tannin or the soluble solids content of the volume of liquor used. The ratio of these two figures is, of course, dependent on the purity of the extract. Because of the highly arbitrary nature of the official methods of tannin analysis, the formaldehyde figure (when expressed as a percentage of the tannin content) is of more importance as a means of classification and identification than as a possible basis for quantitative estimation of individual tannins.

The astringency of a tan liquor is probably its most important single characteristic; and it is found to be dependent on such subsidiary factors as concentration, pH, ratio of tannin to non-tannin, average molecular weight, and degree of dispersion, as well as on the chemical constitution of the tannins and non-tannins present.

While in general it is possible to predict the astringency of a tan liquor (under standard conditions) in rough fashion from a study of the tannin/non-tannin ratio, it has been found that the prediction is sounder if this ratio is considered in conjunction with the buffer index of the liquor.

The buffer index of a tannin infusion, tanning extract, or yard liquor is arbitrarily defined in the publications of the British Leather Manufacturers' Research Association (3, 6) as the number of millilitres of normal sodium hydroxide or hydrochloric acid solution required to change the pH of 100 ml. of liquor of 20° Barkometer strength (20° Bk. = specific gravity 1.020) by one unit within the limits pH = 3.0 to pH = 5.0, which is the range normally encountered in tannery practice. For a given extract, at a concentration equivalent to 20° Bk. strength, the buffer index is inversely proportional to the purity or the tannin/non-tannin ratio; and, as pointed out above, the astringency of a liquor is found to be a complex function of the pH, concentration, and tannin/non-tannin ratio. It is obvious that the astringency of an extract will be more greatly reduced, and the buffer index more greatly increased, if the non-tannin fraction consists of weakly acidic, polyphenolic compounds rather than of sugars or other materials of low buffering power.

For these reasons it was thought that a comparison of the buffer index of the white spruce extract with the corresponding values for commercial extracts of known characteristics would afford some indication of the astringency of the former, it being borne in mind that the spruce extract studied was representative neither of the total extractable matter of the raw bark nor of a potential commercial extract efficiently prepared.

For the determination of the buffer index values, glass electrode titrations and routine analyses (by the A.L.C.A. method, with slow cooling) were carried

out on the white spruce extract, diluted to 20° Bk., and on suitably diluted commercial samples of ordinary (unsulphited) solid quebracho, liquid hemlock, liquid oak bark, and paste gambier extracts.

The formaldehyde precipitation figures for all these extracts, as well as for white spruce, black spruce, and Western hemlock barks, were determined as part of the classification study.

It was originally intended that the scheme of Cameron and McLaughlin (2) be followed in the study of the titration curves of the spruce extract and of the various fractions obtained by steam distillation, ether extraction, and dialysis. However, the spruce extract was unlike the extracts investigated by Cameron and McLaughlin, in that the ether-extractable fractions were not large and their titration curves were not simple; hence the plan was not followed in detail.

Experimental

Extraction Characteristics

Tests were made on the efficiency of extraction at various temperatures, an extractor of the Koch (International) type being used, and the results were compared with extraction by the official methods.

Preparation of Concentrated Extract

A mixed sample of bush-peeled barks, analyzing 21.0% tannin and 16.4% soluble non-tannins (A.L.C.A. method, dry basis), was extracted in a small counter-current diffusion battery of nine 32-ounce bottles; eight were kept in a boiling water bath and one at room temperature.

In spite of the high temperature used, the extraction was slow and inefficient, principally because of the somewhat crude design of the apparatus, but a sufficient quantity of extract of 27° Bk. strength was obtained for the work in hand. This liquor had the following composition: insolubles, 1.92; soluble non-tannins, 29.18; tannin, 41.60 gm. per litre; total 72.70 gm. per litre. The purity was thus 58.8% as compared with 56.1% for the raw bark liquor.

The extract was stored in a refrigerator at 0° C. in order to check fermentation and bacterial action without resort to the use of antiseptics or fungicides; it was later noted that mold growth did not occur in liquors of 20° Bk. or greater strength. The liquor was brought to room temperature only as required for sampling.

Color of Extract

Several color tests on calfskin grain splits were made according to the (then) tentative method of the A.L.C.A. for skin color tests.

Formaldehyde Precipitation Test

In Lauffmann's method (4) the reagent consists of the following: water, 100; 40% formalin, 150; concentrated hydrochloric acid, 100 parts by volume. The reagent (25 ml.) was refluxed for 30 min. with 50 ml. of tannin solution of analytical strength (*i.e.*, containing 4.00 ± 0.25 gm. of tannin per litre). The flocculent precipitate was filtered off hot on a Gooch crucible with asbestos

mat, and washed with boiling water, then dried to constant weight at 105° C. The results are given in Table II.

Bromine Precipitation

On addition of 25 ml. of saturated bromine water to 50 ml. of spruce extract of analytical strength, a flocculent precipitate formed immediately. The precipitate was filtered off only with great difficulty and was redispersed on washing, hence the study was confined to an analysis for bromine content.

TABLE II

FORMALDEHYDE PRECIPITATION FIGURES FOR VARIOUS BARKS AND EXTRACTS

	Formaldehyde figure	
	As % of soluble solids	As % of tannin
Barks—		
White spruce	70.5	124.0
Black spruce	46.5	116.0
Western hemlock	73.9	142.0
Extracts—		
27° Bk. white spruce	72.2	111.0
Ordinary solid quebracho	98.7	114.5
Eastern hemlock	60.4	102.0
Liquid oak bark	6.9	10.6
Paste gambier	83.1	153.0

Steam Distillations

Steam distillation of 100 ml. portions of the original 27° Bk. extract and of the ether-extractable fraction was carried out, apparatus based on that of Cameron and McLaughlin (2) being used.

Ether Extraction

Extractions with ethyl ether were run for a minimum of 72 hr. in a 500 cc. continuous extractor* ((1), Fig. 1). The residual ether-extracted liquor was warmed gently and outgassed with hydrogen to remove traces of ether, then diluted to the original volume.

The ethereal extract separated into two layers because of the water carried over during the long extraction. The proportionately small amount of aqueous solution appeared to contain some tannin, hence it was shaken three times with dry ether; the ether layers were added to the original ether extract, and the aqueous layer to the residual ether-extracted liquor. The ethereal extract was then carefully concentrated on the steam bath to minimize loss of volatile constituents.

Concordant results in regard to the percentage of ether-extractable matter (other than volatile acids, such as acetic) were very difficult to obtain, principally because of retention of traces of solvent on drying at low temperatures and loss of volatile phenolic compounds at higher temperatures. For these reasons the concentrated ether extracts were redissolved in a minimum amount of methanol, then made up to volume with distilled water; portions equivalent to 100 ml. of the original 27° Bk. extract were pipetted out for the titrations or other operations. One such aliquot portion was titrated after steam distillation, others were titrated after drying at 25° and 105° C. and redissolving in water.

* *As supplied by the Scientific Glass Apparatus Company of Bloomfield, N.J., U.S.A.*

Titration Curves and Buffer Index Values

The circuit used in the glass electrode titrations was designed by V. G. Smith ((7), Fig. 1) and built by the senior author. It included the highly sensitive General Electric F.P.–54 Pliotron tube in conjunction with a Leeds and Northrup Type K2 potentiometer and Type 2420-D galvanometer.

The glass electrodes consisted of bulbs of Corning 015 glass blown on to stems of ordinary soda-lime tubing. The electrode system included a saturated solution of quinhydrone in 0.1*N* hydrochloric acid, a platinum wire being used as the internal electrode; this wire was sealed through the end of a smaller tube filled with mercury for the external connection.

The reference electrode was a small saturated calomel half-cell, so designed that it could be used with either the calibration or titration vessel. The bridge solution in both vessels was saturated-potassium-chloride–calomel.

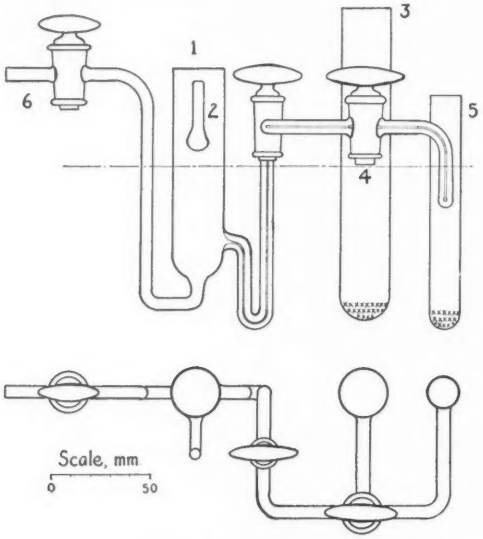

FIG. 1. *Calibration vessel for use in oil bath.* 1, *Hydrogen or glass electrode cell;* 2, *hydrogen vent;* 3, *saturated potassium chloride reservoir;* 4, *three-way capillary stopcock;* 5, *calomel electrode cell;* 6, *suction tube for flushing out hydrogen electrode cell.*

The calibration vessel, Fig. 1, was designed for use in an oil bath, and could be adapted rapidly to hydrogen, quinhydrone, or glass electrode measurements. Despite the unorthodox arrangement of the liquid junction, measurements of potential with stable buffers were reproducible to within a few tenths of a millivolt. Because of the relatively high temperature coefficient of the saturated calomel half-cell, all calibrations and titrations were carried out at 20.0 ± 0.1° C. in a well stirred kerosene bath, which was found to be quite

as convenient as a water bath, and was, of course, essential because of the characteristics of the electrometer circuit.

The titration vessel, Fig. 2, was constructed from an ordinary bottle (70 mm. inside diameter). The motor-driven Bakelite stirrer, the dipping arm of the calomel half-cell, the glass electrode, and a 10-ml. microburette passed through holes bored in the varnished stopper.

With the circuit and calibration vessel described it was possible to obtain calibration curves for the glass electrodes (buffers accurately standardized by means of the hydrogen electrode being used) which were nearly linear from pH = 1.5 to pH = 12.0; the slope of the curves was usually within 1 to 2% of the theoretical value, *i.e.*, 0.0001983 T, where T = temperature in °K. A group of typical calibration curves for the glass electrodes is shown in Fig. 3.

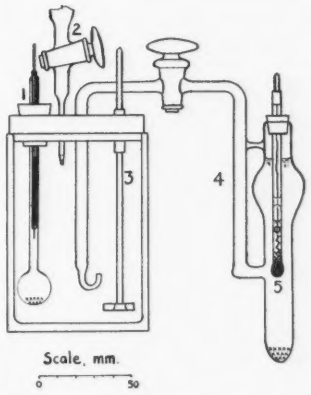

FIG. 2. *Titration vessel.* 1, *Glass electrode;* 2, *10 ml. microburette;* 3, *Bakelite stirrer and glass bearing;* 4, *saturated potassium chloride bridge;* 5, *calomel electrode.*

In the titrations, the standard, approximately normal, solutions of sodium hydroxide and hydrochloric acid were added in small portions from the microburette, sufficient time being allowed after each addition for the attainment of equilibrium conditions before the final potential measurement was taken.

The analyses, buffer index values, and other relevant data for the white spruce and commercial extracts of 20° Bk. strength are given in Table III, and the curves from which the buffer index values were calculated are plotted in Fig. 4. In each case the calculation involved merely reading off the total volume of 1.0N acid and alkali required to change the pH from 3.0 to 5.0; change in the slope of the curves within these limits was ignored.

TABLE III

ANALYSES AND BUFFER INDEX VALUES OF 20° BK. LIQUORS

——	Quebracho	Spruce	Hemlock	Gambier	Oak bark
Analysis, gm. per litre—					
Insolubles	1.03	1.80	0.95	3.80	2.00
Soluble solids	49.80	51.00	49.30	48.70	45.30
Soluble non-tannins	6.93	20.15	20.08	23.20	15.60
Tannin	42.87	30.85	29.22	25.50	29.70
Purity, %	86.1	60.4	59.3	52.4	65.5
Tannins/non-tannins	6.19	1.52	1.46	1.10	1.90
Initial pH	4.32	4.00	3.71	4.32	2.75
Buffer index	0.55	1.09	1.72	1.78	2.15

In Fig. 5 are given the titration curves for the undiluted 27° Bk. spruce extract and the various fractions mentioned above: the steam distillate, aliquots of the ether-extractable material, and the ether-extracted liquor itself. The curves for the steam distillate and ether-extractable fractions are replotted on a larger scale in Fig. 6.

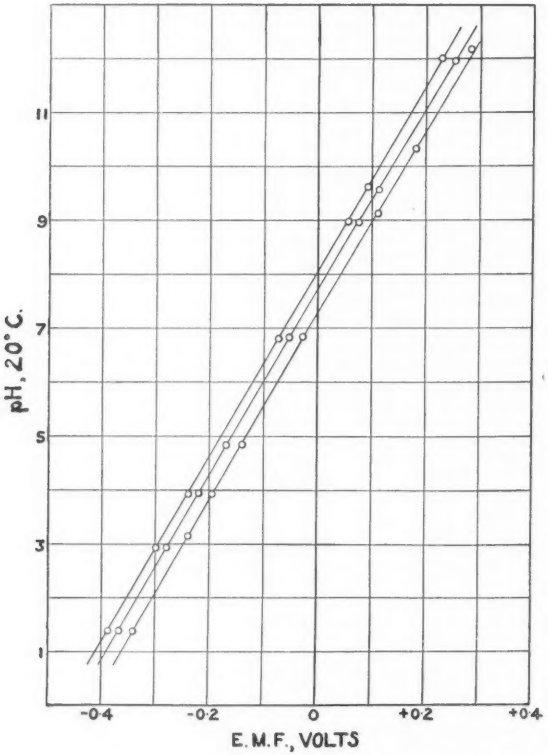

FIG. 3. *Typical calibration curves for Corning 015 glass electrodes, with 0.1 normal-hydrochloric-acid–saturated-quinhydrone filling, and saturated calomel half-cell, at 20° C.*

Fermentation

A portion of the original 27° Bk. spruce liquor was allowed to ferment freely at room temperature, since the fermentation of an extract is of importance to the tanner. Titrations of 100-ml. portions were carried out in the usual way.

Later, examination showed that the fermentation, which was marked by evolution of gas and formation of alcoholic materials, was due to two or more varieties of wild yeast, rather than to bacteria or molds.

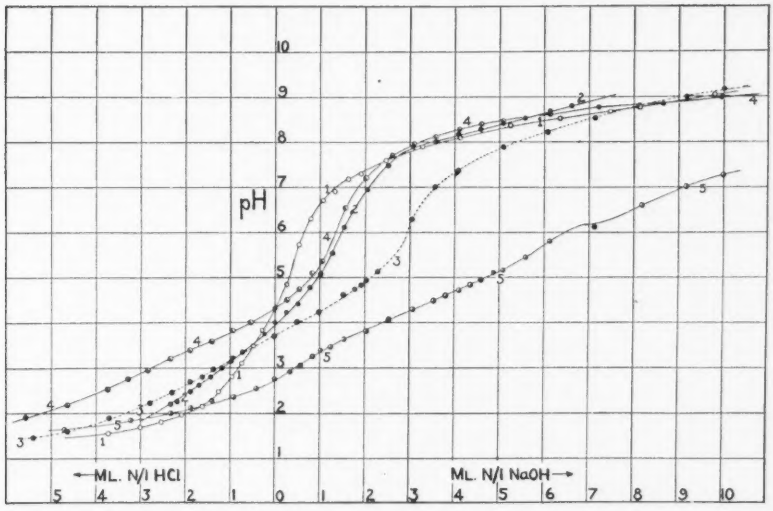

FIG. 4. *Titration curves for 100-ml. portions of 20° Barkometer liquors.* 1, *Ordinary quebracho (solid extract), open circles, full line;* 2, *white spruce extract, solid circles, full line;* 3, *liquid hemlock extract, solid circles, dotted line;* 4, *paste gambier, half-black circles, full line;* 5, *liquid oak bark extract, half-black circles, full line.*

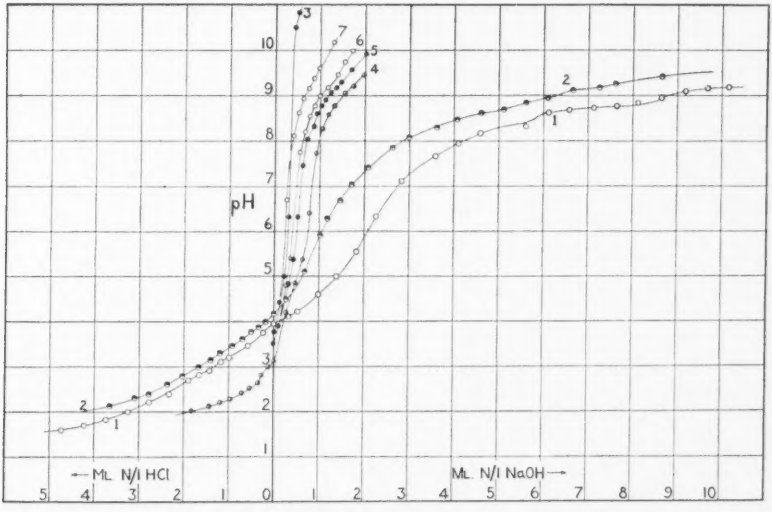

FIG. 5. *Titration curves for original and ether-extracted 27° Barkometer spruce extract (100-ml. samples), and for steam distillate and ether-extractable fractions from 100-ml. samples of original liquor.* 1, *Original 27° Bk. extract;* 2, *same after ether extraction;* 3, *steam distillate;* 4, *total ether extract;* 5, *same after steam distillation;* 6, *ether extract after drying in vacuo at 25° C.;* 7, *same after drying at 105° C. (See Fig. 6).*

Discussion of Results

It is evident from the formaldehyde precipitation data that the tannins of white and black spruce bark are typical examples of the catechol or condensed group, in Freudenberg's classification. The bromine content of the bromo derivative of white spruce tannin is 52%, which is in agreement with values for other tannins of this group given by Perkin and Everest (5).

Tests on calfskin showed that the color was quite satisfactory; the samples had colors somewhat similar to those of samples tanned with sulphited quebracho extract.

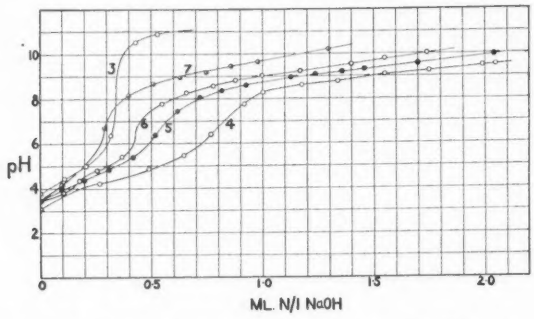

FIG. 6. *Titration curves for steam distillate from 100 ml. of 27° Bk. liquor (Curve 3); total ether extract from 100 ml. (Curve 4), and same after steam distillation (Curve 5); ether extract from 100 ml. after drying in vacuo at 25° C. (Curve 6), and after drying in air at 105° C. (Curve 7).*

The buffer index and tannin/non-tannin ratio of the white spruce extract indicate that the liquor would probably be, in its general tanning properties, intermediate between the highly astringent extracts, such as ordinary quebracho and mimosa, on the one hand, and milder extracts such as hemlock, oak bark, and gambier, on the other. Certain chestnut wood extracts of French manufacture (3) show on examination both tannin/non-tannin ratios and buffer index values approaching those of the white spruce liquor, but information regarding their tanning properties is not available at the time of writing, hence it would be unwise to attempt any extensive predictions of the behavior of the spruce extract in the tannery.

The high buffer index of the oak bark extract examined is undoubtedly due in part to acidic fermentation products, since the pH of the undiluted liquor fell from 3.25 to 2.75 during the five months preceding this study. The pH of the hemlock extract fell from 4.20 to 3.71 during the same period. This acid-forming or "souring" tendency is of real importance in the rocker section of the heavy leather yard. The pH of a portion of the 27° Bk. spruce extract fell from 4.00 to 3.84 when it was allowed to ferment freely at room temperature for two months. It was found that the fermentation was due to two or more varieties of wild yeast and not to bacteria or molds. As expected, the titration curve of the fermented sample did not deviate greatly from that of the original material. The rapid fermentation caused by yeasts indicates

that the non-tannin fraction contains an appreciable proportion of sugars, which under tannery conditions might ferment to lactic, acetic, and other acids.

The titration curves for the steam distillate and the ether-extractable fractions (Figs. 5 and 6) suggest the presence of a number of weakly acidic (phenolic) materials in addition to volatile acids. The ether-extractable material not volatilized at 105° C. constitutes about 6.4% of the weight of soluble solids in the liquor, but its low buffering power (Curve 7, Fig. 6) indicates that it does not consist in large part of gallic acid or similar compounds. The volatile acids content of the unfermented 27° Bk. liquor was equivalent to about 0.18 gm. of acetic acid per litre.

A comparison of the curves for the original and ether-extracted 27° Bk. liquor shows that about 19% of the buffering power of the former (in the range pH = 3.0 to 5.0) is due to the ether-extractable material and the acids volatile with steam.

The ease of extraction of the tannins of white spruce bark is a matter of importance from several points of view. Tests made with an extractor of the Koch type at various temperatures showed that 76.2% of the tannin and 71.9% of the soluble non-tannins, these values being referred to the corresponding figures as found by means of the I.S.L.T.C. method of analysis as 100%, are ultimately extractable with water at 25° C. Accordingly, immersion in river water or leaching by rain or melting snow would cause serious losses of tannin. Thus, for example, analysis by the A.L.C.A. method of a sample of white spruce bark obtained from logs at a pulp mill revealed a tannin content of only 4.2%.

In conclusion it may be said that if concentrated tanning extracts can profitably be prepared from white spruce bark, then it is probable that these extracts could, because of their astringency, be used only in admixture with milder tanning materials in heavy leather manufacture, but might be used alone where straight quebracho extracts are now used, *e.g.*, in drum tannages of sheepskins, insole splits, and others.

Acknowledgments

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